PART I

INTRODUCTION

CURRENT STATUS OF THE SUBJECT

Recent advances in molecular biology of bacteria and bacteriophages have greatly facilitated efforts towards elucidation of gene regulation in higher organisms. In these endeavours, much attention is being focussed on the nucleus and its constituents as this is the main repository of the genetic material in the cells of higher organisms. Of the many functions of the cell nucleus, one of the most important is the process of gene regulation whereby information encoded in the DNA is utilized in the synthesis of complementary RNA molecules. It is through these moieties that the genetic information is ultimately transmitted to the protein synthesising machinery in the cytoplasm, where it is decoded for the synthesis of specific proteins.

Precise controlling of the transcription of specific genes is extremely important both during the development and normal functioning of cells of higher organisms. For example, differentiated cells that perform different functions utilize characteristic sets of genetic information and so must use different regions of the genome for the synthesis of RNA. Since the DNA of each cell in a metazoan appears to be the same, it may be supposed that various cell types may be differentially restricted in the utilization of genetic information contained in their DNAs. Specific regulatory mechanisms must therefore be available for activating and inactivating particular regions of the genome for RNA synthesis, depending on the characteristics of the cell. The mechanism by which this selection and regulation of genetic potential is accomplished in higher organisms is still largely unknown and presents one of the most challenging problems in modern biology.

It is proposed here to survey the current status of the problems of eukaryotic transcription. The survey will also briefly cover current information on the compositional and structural aspects of eukaryotic genome as this knowledge is of direct relevance to the mechanism of transcription.

Structure and composition of interphase eukaryotic chromosomes

From the point of the view of eukaryotic transcription, the knowledge of composition and structure of chromosomes of the cells in interphase stage is of utmost importance. Unlike bacterial chromosome, which is composed primarily of naked DNA, the eukaryotic chromosome is a complex structure (1). The eukaryotic chromatin (the chromosomal material extracted from the nuclei of cells of higher organisms) contains besides DNA, large amounts of histones, nonhistone proteins and small amount of RNA in approximate proportions of 1:1:0.5 - 1.2:0.05 respectively (2). Histone polypeptide contain large amounts of basic amino acids in contrast to nonhistone proteins which are acidic in character. Unlike histones, which maintain the ratio (1:1) with DNA in different cells, nonhistone proteins vary in the ratio with DNA in cells in different stages.

All the components of chromatin have been implicated in the regulation of transcription. Current information on the structural features of these elements will be summarized here in order that the parts played by them in the chromatin function are better understood.

DNA:

The linear morphological variation evident in somatic, meiotic lampbrush and salivary gland chromosomes seems to suggest that chromosomes

are similarly differentiated in a genetic sense, that is the genes which differ among themselves in phenotypic expression, are organised along the length of the chromosomes in linear fashion. Experimental evidence favours the unineme chromatid theory which postulates that a single double-helical DNA molecule runs along the entire length of chromosome (3).

The number of chromosomes in the haploid genome of different organisms may vary widely and one can hence suppose that there are as many pieces of linear DNA helices differing in nucleotide sequences per eukaryotic genome. The total length of such DNAs could range anywhere from 100 to 1000 times that of prokaryotic DNA (E.coli DNA is about 1000 µ in length (4)).

Renaturation kinetics indicates that chromosomes of most eukaryotic cells consist of unique and reiterated nucleotide sequences (5) and this heterogeneous collection of different families of DNA can also be distinguished based on differential sedimentation in centrifugal speeds, timing of replication and differences in base composition. The highly repetitious regions of DNA, satellite DNA, is present in almost all eukaryotes. This may occupy 1-30% of the whole DNA and usually contains 10^5-10^6 repetitive sequences per genome (6). The satellite DNA is concentrated mainly in heterochromatin and near centromere of metaphase chromosomes (7). Schildkraut and Maio (8) showed that the nucleolus is also enriched considerably with this DNA. With the exception of cistrons coding for r-RNA (9) and histone m-RNA (10), it has not been possible to detect RNAs synthesized in vivo complementary to satellite DNA (11). The unique regions of DNA fraction do not possess

the reiterated base sequences and are thought mainly to constitute structural genes (12). To-date, it appears that *in vivo* synthesized unique RNA hybridizes with about 2-3% of total DNA in the eukaryotic cell (13,14). There is also another class of reiterated sequences termed as intermediate or kinetic fraction. These sequences are less multiplied and renature more slowly than satellite DNA (15). This fraction represents about 37% of the whole genome (15,16).

Histones:

Histones are major structural proteins of chromatin and found in chromosomes of all eukaryotic somatic cells. They are basic and of relatively low molecular weight [10000 to 21000 daltons (17)]. Based on the content of the basic amino acids (arginine, lysine and histidine) histones have been broadly placed into five distinct classes: H_1, H_2A, H_2B, H_3 and H_4 as per the nomenclature agreed at the Ciba symposium (18) [or F_1, F_2a_2, F_2b, F_3 and F_2a_1, as per John's nomenclature (19)]. Further attempts at sub-fractionation reveal that each of the classes contain only a limited number of different polypeptides. In recent years, complete amino acid sequencing of some histone components has been achieved (18). These studies have revealed that the histones are very highly conserved proteins, there being very little variation in the evolutionary scale (18). On the basis of this knowledge, histones have been implicated to have a fundamental role in chromatin structure and possibly in its function which will be discussed later. The histones are however among the most highly modified proteins.

The modifications include acetylation (20,21), methylation (20,21) and phosphorylation (22). Since the amino acid sequences are so highly conserved, such changes are likely to have significant effects on chromatin structure (23).

In the developing sperm cell, the histones characteristic of the somatic cell are entirely displaced from their combination with DNA by a new series of small (mol. wt. 6000 daltons). highly arginine-rich sperm-specific polypeptides termed as the protamines (24,25). These proteins seem to be absent in Neurospora crassa (26) and Microsporum gypsum (27).

Nonhistone chromosomal proteins:

Unlike histones, nonhistone protein fraction displays a considerable heterogeneity as seen from a wide spectrum of molecular weights (5000 to 100000 daltons) (28-30) and from electrophoretic separation into more than 25 polypeptide chains (31,32). A number of well-defined enzymatic activities are found associated with these proteins as is apparent from the list given in Table 1. These proteins are enriched in euchromatin region known to be active in RNA synthesis (62). Since the nonhistone proteins exhibit considerable heterogeneity and variability, one important roles for these proteins in the regulation of gene expression has been suggested. This aspect is discussed again under mechanism of eukaryotic transcription.

Chromosomal RNA:

Chromatin has been reported to contain a small amount of RNA. This RNA is smaller in size (about 3.2 S), is enriched with a unique

Table 1

	Nonhistone protein-Associated Enzymes	Reference
1	Adenosine triphosphatase	(33)
2	Deoxyribonuclease, alkaline	(34)
3	Deoxyribonuclease, neutral	(35)
4	DNA polymerase	(36, 37)
5	Glutamate dehydrogenase	(33)
6	Glutamic - oxaloacetic transaminase	(33)
7	Histone acetylase	(38, 39)
8	Histone methylase	(40, 41)
9	Histone phosphokinase	(42)
10	Histone protease	(43)
11	Lactate dehydrogenase	(33)
12	Malate dehydrogenase	(33)
13	Nucleases	(43, 44)
14	NAD glycohydrolase	(45, 46)
15	Nucleoside triphosphatases	(47)
16	Protease, Neutral	(48)
17	Protein phosphokinase	(39, 49)
18	Poly ADP-ribose synthetase	(46)
19	RNA methylase	(50)
20	RNA polymerase	(51 - 57)
21	RNA terminal transferase	(58)
22	Polynucleotide ligase	(59)
23	Histone deacetylase	(60)
24	Nonhistone protein kinase activities	(61)

base - dihydro-uridine (51,63,64) - and is covalently linked to certain nonhistone proteins. Bekhor et al. suggested that this RNA may play a regulatory role in transcription (65). Several workers have however failed to detect this RNA species (66-68).

Structural features of chromatin

A number of studies have been carried out on the structure of interphase chromatin. It has been suggested that chromatin is a smooth linear fibre consisting of a regular (69) or irregular (70) supercoiling of the histone-bonded double helix. Recent results have however revealed some novel features of chromatin structure. Evidence emanating from electron microscopy (71-73) indicates that a substantial portion of the chromatin is composed of fibres containing beads (termed as 'nu' bodies) with an average diameter of about 80 Å (71,74). This has also been inferred from the experiments based on controlled cleavage of chromatin by certain nucleases which yield nucleoprotein particles similar to the 'nu' bodies. Recent work has thrown more light on the nature of these particles. They have been found to contain a DNA segment of about 200 base pairs associated with histones (75-80). Taken together, the recent studies favour the model of 'beads on the string' for chromatin structure in which the DNA is visualized to fold around histone complexes spaced regularly along the chromosomal fibre (81-83). The repeating unit is suggested to be composed of two each of H_4, H_3, H_2A and H_2B histone molecules and DNA strand of about 200 base pairs (71,74-80, 82-84). The histones are envisaged to exist as complexes formed by

interaction of C-terminal halves, leaving N-terminal regionsfree to interact with DNA; these complexes can serve as cores upon which DNA can be wound (85). Histone H_1 may not be an integral part of the beads and it is proposed that this protein may be a cross-linking agent between 'nu' bodies (85). Nonhistone proteins may not form the part of the basic structure responsible for the compaction of the DNA in chromatin. A number of questions on chromatin structure still remain to be resolved. Perhaps the most outstanding among these is: structural differences between genetically active and inactive chromatin regions. From the recent experiments of Bonner et al (56) it appears that both the active and inactive chromatins contain complexes resistant to nuclease (endonuclease DNase II). The nuclease resistant structures of inactive chromatin have been found to be DNA-histone complexes ('nu' bodies) sedimenting at 11-13 S while those of active chromatin are complexes of DNA, RNA, histone and nonhistone proteins sedimenting at 14 and 19 S (86).

The problem of chromatin structure is closely related to its mode of replication. In recent years, a great deal of attention has been focussed on the mode of replication of eukaryotic chromosomes. The basic features of eukaryotic chromosomal replication seem similar to prokaryotic DNA replication. The process seems to be complicated owing to the enormous size of eukaryotic DNA. DNA replication in eukaryotes appear to start at many points; DNA synthesis may begin simultaneously at these points (87-90) but may finish at different times within S-phase, depending upon the lengthsof replicons. Evidence based on autoradiography and sedimentation studies reveals that eukaryotic DNA like E.coli DNA, is synthesised first as short pieces

(Okazaki fragments) and then joined together to form larger DNA molecules (91). The synthesis of both histones (92,93) and nonhistone proteins (94) of chromosomes (which are believed to be synthesized in cytoplasm) are in some fashion intimately interlinked with the synthesis of DNA. Indeed, there are reports to suggest that synthesis of histones may be essential for stabilizing the newly formed DNA (95). Elucidation of these mechanisms should give further insights into the structural features of chromatin.

Mechanism of eukaryotic transcription

Spectacular advances made in recent years in transcription in prokaryotes have greatly helped similar pursuits in eukaryotic organisms. It would be pertinent for the present discussion to briefly review some salient findings of prokaryotic transcription research emanating mainly from studies with E.coli and its phages (96). The transcription sequence could be broken down into following steps:

(i) Binding of RNA polymerase to DNA (presumably to the promoter regions of DNA) (2); (ii) initiation of RNA synthesis (needs σ factor for specific initiation and inhibited by rifampicin) (97); (iii) elongation of the RNA chains (inhibited by low concentration of actinomycin-D) (96,98); and (iv) termination or cessation of RNA synthesis. This step requires and additional protein factor called ρ factor (96,98).

Bacteria seem to have a single RNA polymerase. The E.coli RNA polymerase is the best characterised so far consisting of 5 polypeptide subunits β, β', σ, α and ω with molecular weights of 160000, 150000, 90000, 40000 and 12000 daltons respectively (18, 99). The σ subunit seemsto perform the role of a specific initiator of operons.

Unlike in bacterial cells, eukaryotic cells possess multiple forms of RNA polymerases which seem to be tightly bound to chromatin (100,101). The RNA polymerases are separated mainly into three classes on the basis of chromatographic patterns, activities in the presence of divalent cations and susceptibility towards specific inhibitors (102,103). Type I (or Class A) RNA polymerase is of nucleolar origin while Type II (or Class B) and Type III (or Class C) are nucleoplasmic. Type II RNA polymerase is involved in the synthesis of DNA-like RNA, is activated by Mn^{++} and inhibited by α-amanitin (104). The overall pattern of the eukaryotic enzymes resembles that of prokaryotic enzymes, since each enzyme comprises two subunits of high molecular weight (190000 and 135000 daltons) and several smaller ones (48000, 44000, 37000, 29000, 24000, 20000, 16000 and 14000 daltons) (100). The structural and immunological studies suggest that some subunits could be common to the Type I, II and III RNA polymerases but it is quite likely that most of the subunits of the three enzymes are the products of distinct genes (100).

The other component of eukaryotic transcription machinery - the chromatin template - is presumably endowed with additional control devices not encountered in prokaryotic transcription. It is known that chromatin is much less active as template than its DNA and this seems to be due to the presence of histones (105-107). Chemical modifications of histones in vivo, namely, acetylation, methylation and phosphorylation - which probably weaken the binding of histones to DNA (108-113) - have been suggested to cause enhancement in chromatin template efficiency. The evidence for this presumption stems from studies, among others, on

hormone-induced gene activation in animal tissues (114,115). Langan suggests that stimulation of RNA synthesis by the hormones that increase the concentration of cyclic AMP in the respective target tissues could be due to cyclic AMP-induced stimulation in the rate of histone phosphorylation (114,115).

The involvement of various histone components in transcription needs to be carefully examined on the basis of recent knowledge on chromatin structure discussed earlier. The levels of histones in active and inactive regions of chromatin (euchromatin and heterochromatin respectively) have been found to be similar(18). In view of this finding and the fact that only a few different molecular species of histones exist, it has been postulated that histones may not act as specific gene repressors like the bacterial repressors but they may have a general inhibitory effect on transcription (116).

Possible involvement of nonhistone proteins in gene regulation is implicated in a variety of observations. Unlike histones, nonhistone chromosomal proteins are considerably heterogeneous (117) and also they are found in higher amounts in the active regions of chromatins as compared to the inactive region (117). The proteins exhibit rapid turnovers which (in contrast to histones) are totally unrelated to chromosomal replication (18). Under the conditions which lead to gene activation, turnovers of these moieties exhibit further acceleration (117). Interestingly under different conditions of gene activation, syntheses of different nonhistone proteins are found to get selectively stimulated (30,118-124). Chemical modifications of nonhistone proteins,

such as phosphorylation, are also implicated in gene activation (1,18). It is suggested that interactions of phosphorylated nonhistone proteins with histones may cause the weakening of DNA-histone binding and in turn, raise template activity of chromatin (125).

Recent studies have attempted to get deeper insights into the mechanisms of eukaryotic transcription. It has been shown that RNA chain initiation sites (or RNA polymerase-binding sites) on mammalian chromatin are about 1/10th those available for corresponding DNA whereas the rate of movement of RNA polymerase along chromatin is only about 1/3rd that along corresponding DNA (126). The blocking of the RNA chain initiation sites (presumably in selective manner) seem to be a major role played by the non-DNA components of chromatin in regulation of transcription. It appears that only homologous RNA polymerases identify the correct RNA chain initiation sites. Thus it has been demonstrated that the number of initiation sites on calf-thymus chromatin is much greater with *E.coli* RNA polymerase than with calf-thymus RNA polymerase (126). It has been suggested that like in prokaryotic transcription, proteins having specific roles in eukaryotic transcription may be involved. Existence of factors involved in initiation (127,128), elongation (129-132) and termination (127) of RNA chain growth has been reported. It seems that some of these belong to nonhistone chromosomal protein fraction (133).

Processing and intracellular transport of RNAs

This survey on the current status of regulation of eukaryotic RNA synthesis will not be complete unless current research on the

steps intervening transcription and translation are not mentioned here. Unlike in prokaryotes, transfer of genetic information in eukaryotes constitutes not only the transcription and the translation, but also the processing of transcribed RNAs and their transportation from the nucleus to the cytoplasm. Details of these steps are beginning to be understood only in recent years. In many eukaryotes, 28S and 18 S RNAs of the two ribosomal subunits (60 S and 40 S) have been shown to arise from a 45 S RNA precursor synthesised in the nucleolus (134).

Transfer RNAs are formed from a precursor molecule which is 20 to 30 nucleotides longer than tRNA. The pre-transfer RNA synthesised in the extra-nucleolar region seems to be exported quickly to the cytoplasm, then methylated and processed to appropriate sizes (134,135).

The messenger RNAs are suggested to be synthesised in the nucleus as parts of giant heterogeneous nuclear RNAs (134). These messenger RNA precursors have wide range of sizes from about 10-100 S, whereas mRNA sizes range from 10 to 20 S (136, 137).

The elaborate ways by which RNAs are processed in eukaryotic cells would imply that in addition to the regulations at transcription and translation levels, protein synthesis in these cells could be controlled by modulations in the processing of RNA precursors and their intracellular transport.

Current models for regulation of eukaryotic transcription

The Jacob-Monod model of regulation, based on the analysis of β-galactosidase induction in E.coli, has been the directing force for the current concepts on the regulation of gene expression in higher

organisms (138). A number of refinements have been made in the original model from time to time and efforts are being directed to get clearer understanding in molecular terms. Of particular significance in this regard are the researches aimed at elucidating interactions between RNA polymerase and promoter regions of template DNA. Chamberlin (139) has proposed that the RNA polymerase on encountering a promoter region recognises the sequences in it to form a primary complex. This complex is transformed into another complex in which the DNA strands are separated and the enzyme made accessible to the template strand. Formation of RNA chain is then initiated from the open complex in the presence of ribonucleoside triphosphate precursors (126).

In attempting to postulate regulatory mechanisms of eukaryotic transcription, these facts need to be taken into account : (i) the size of eukaryotic DNA is very much greater than that of prokaryotic DNA; (ii) the proportion of the region in the total eukaryotic DNA which contains utilisable genetic information is small (140); (iii) there is a great deal of reiteration in eukaryotic DNA; (iv) proteins in chromosomes greatly influence transcribability of the DNA to which they are associated (18,116); and (v) eukaryotic messenger RNAs have enormously longer precursors, the non-messenger RNA portions of these do not leave the nucleus (134).

Many models for regulating gene transcription in eukaryotes have emerged recently which explain some of the facts listed above and have certain amounts of experimental evidence. The models have

retained the basic tenets of prokaryotic transcription comprising regulating and structural elements of an operon. The models proposed by Britten and Davidson (141), Georgiev (142), and Scherrer and Marcaud (143) try to account for many of the properties of HnRNA, chromosomal proteins and the presence of unique and reiterated sequences in DNA. A model for gene regulation in eukaryotic cells has been recently suggested by Monahan and Hall (144). This model proposes two major elements controlling gene transcription in eukaryotic cells : an RNA element (derived from noninformative part of HnRNA) turning on genes and a protein element turning them off. The essential feature of this model is that of having a series of interlocking elements for selective activation or restriction of structural genes. Conformational features of chromosomes assume significance in the mechanisms of eukaryotic transcription suggested by Crick (145). According to him, chromosomal DNA falls into two classes, namely, 'fibrous DNA' which constitutes structural genes and 'globular DNA' which includes the recognition site for regulating the transcription of structural gene. Within the globular regions are twisted hairpin loops of double-stranded DNA which, in view of the geometry involved, will come apart into two single-stranded chains. It is envisaged that RNA polymerase specifically binds to these regions and moves out to transcribe along fibrous DNA regions. This prediction appear to be consistent with much of the newly emerging picture on chromosome structure discussed earlier.

SCOPE OF THE WORK TO BE REPORTED

The foregoing literature survey serves to demonstrate that eukaryotic transcription is much more complex than prokaryotic transcription and this is further so in multicellular organisms. However, a beginning has been made towards elucidation of regulatory aspects of transcription in higher organisms. The voluminous new information that is being added to the subject by a variety of investigations has no doubt helped unravelled many intricacies of eukaryotic transcription. It has now been well recognised that adaptations by a higher organism to many internal and external provocations are achieved primarily through subtle modulations in transcription in crucial cells. Such stimuli can be profitably harnessed to understand several finer controls in eukaryotic transcription. In studies to be presented in the thesis, attempts have been made to analyse a number of molecular events postulated to be involved in eukaryotic transcription using, as a system, the liver of rats subjected to widely differing stress conditions: (i) whole-body exposure of animals to ionising radiation, and (ii) partial hepatectomy. The salient findings have been already outlined in the Synopsis.

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