

ACTIVATION OF CHROMATIN

Chromatin structure

The lowest level of DNA coiling in chromatin is the nucleosome, a repeating subunit comprised of a nucleosome 'core' particle of approximately 166 bp of double-stranded B-form DNA wrapped into two left-handed super-helical turns around the outside of an octamer of histone proteins¹. Nucleosomes are arranged into a 10 nm chromatin fibre as 'beads-on-a-string' with 0–80 bp of linker DNA between core particles. Nucleosomes are present on both transcriptionally active and inactive chromatin, but may undergo changes during conversion of one to the other, as discussed in the next section. The bulk of chromatin *in vivo*, especially the inactive chromatin, consists of 30 nm thick fibres. These result from the winding of the 10 nm fibre into a 'solenoid' with approximately six nucleosomes per turn, requiring histone H1 for its stabilization. It is postulated that the 30 nm fibre becomes unfolded *in vivo* when a gene becomes transcriptionally active. Direct evidence for this is based on the sedimentation rates of active and inactive globin gene fragments during sucrose density centrifugation². The third level of chromatin structure appears to be the folding of the 30 nm solenoid into loops or domains of chromatin containing 35–100 kb of DNA³. As with lower levels of chromatin organization, chromatin domains also appear to be dynamic structures and may be related to units of transcription or units of replication.

Active chromatin

(a) *Nuclease digestion studies*: The chromatin of active genes is functionally defined by its increased susceptibility to various endonucleases as compared to the chromatin of inactive genes. The most commonly used endonuclease in chromatin studies is pancreatic DNase I. Micrococcal nuclease and spleen DNase II have been used to a lesser extent. Typical

experimental procedures involve controlled digestion of nuclei by DNase I, followed by isolation of total DNA and probing with specific genes by Southern hybridization procedures. Weintraub and Groudine⁴ were the first to show that the globin gene is preferentially sensitive to digestion by DNase I in chicken erythrocyte nuclei whereas the same gene is resistant to the enzyme in oviduct nuclei, where it is inactive. On the other hand, the ovalbumin gene is preferentially sensitive in chicken oviduct but not in red blood cells. Subsequent studies with several genes have shown that the enhanced DNase I sensitivity of an active gene correlates with the tissue in which the gene is normally expressed⁵.

A second type of nuclease sensitivity is seen at the 5' or 3' ends of most active or potentially active genes, also in a tissue-specific manner. These DNase I hypersensitive sites are ten times more sensitive to DNase I than the corresponding coding regions. For the chick α - and β -globin genes and certain genes in the early embryonic stages of *Drosophila*, the 5' hypersensitive sites are extremely stable and once established, are propagated to daughter cells in the absence of transcription. On the other hand, for several genes such as the sea urchin embryonic histone genes and chick vitellogenin genes, these sites disappear after transcription has ceased. Although the structure or precise function of nuclease sensitive sites has not been firmly established, the hypersensitive sites appear to represent small stretches of control regions of DNA which can interact with regulatory molecules (proteins). Appearance of hypersensitive sites has been correlated with gene activity for several genes such as the herpes simplex viral thymidine kinase gene, mouse metallothionein I gene and *Drosophila* glue gene. In the last example, elegant studies on the *Sgs 4* glue gene have demonstrated that certain *Drosophila* mutants which underproduce the glue protein during embryonic development have deletions in 5' sequences and a loss in DNase I hypersensitive sites.

The molecular basis for the nuclease sensitivity of chromatin is not clear. Earlier electron microscopic studies had demonstrated the absence of regular nucleosomes on the highly active and nuclease sensitive ribosomal genes, suggesting that nuclease sensitivity might be due to the absence of nucleosomes on other genes also³. However, it is now evident that active non-ribosomal genes do have nucleosomes, although these may be in an altered form. Apparently, the DNA associated with nucleosomes is accessible to other molecules such as nucleases and polymerases as it is wrapped around the outside of the nucleosome. A further question is whether DNase I sensitivity results solely from the altered conformation of individual nucleosomes or also involves higher order chromatin structure. In support of the latter, the active ovalbumin gene in chicken oviduct has been shown to exist in a DNase I sensitive domain approximately 100 kb in length, which includes non-transcribed flanking regions also⁶. In the case of the α - and γ -globin genes in chicken erythrocyte nuclei, the flanking 20–50 kb regions are moderately DNase I sensitive as compared to the highly sensitive coding regions⁴; again suggesting a more relaxed higher order structure for active genes. A recent study using procedures of DNA transfection into cultured mammalian cells has reported that a unit of three genes (herpes simplex viral thymidine kinase gene and human α - and γ -globin genes) can integrate into chromosomes and be coordinately regulated⁷. This unit, 20 kb long, behaves as a domain of chromatin and the entire region adopts either a DNase I sensitive or insensitive structure depending on culture conditions. Such techniques for precisely controlling chromatin structure and expression of genes *in vivo* should be of immense use in the future.

At present, nuclease sensitivity of an active gene can be correlated with various changes in the structure and composition of chromatin, such as undermethylation of the DNA, conformational changes in DNA structure, hyper-

acetylation of histones, and association of active nucleosomes with certain non-histone DNA binding proteins of the high mobility group. These are discussed in the following sections.

(b) *Modifications in DNA composition and structure*: There is considerable evidence that changes in DNA composition and structure can be correlated with gene activation. In many higher eukaryotes, an inverse correlation has been observed between gene activation and methylation of cytosine at –CpG– sequences⁸. For the active globin, metallothionein, ovalbumin, γ -crystallin, and various integrated viral genes, there is a tissue-specific pattern of methylation, with the majority of undermethylated sites at the 5' ends of active genes. Direct transfection of methylated and unmethylated cloned genes into cultured mammalian cells has demonstrated that methylated genes (especially methylated at the 5' end) are not transcribed, indicating that undermethylation is a prerequisite for transcription. Undermethylation of a gene has been correlated with DNase I sensitivity for several genes. However, *in vivo* correlations between methylation and gene inactivity are not always absolute (for example, methylated SV40 and polyoma virus DNAs and *Xenopus* ribosomal genes are highly methylated but still active). Part of the reason is that methylation at only 20–30% of the possible –CpG– sequences can be detected by endonuclease digestion and Southern hybridization techniques, and important sites of demethylation may not be detected. Recent advances in genomic sequencing should permit a more thorough analysis of methylation patterns. It is evident, however, that undermethylation is not always sufficient for gene activation. For example, the globin gene is undermethylated in placenta and certain tumour cell lines but is not actively transcribed.

A possible role for 5-methylcytosine is to act as a binding domain for specific regulatory molecules. Another possibility is that DNA methylation could stabilize certain alternate DNA structures. It has been shown that

methylation of the synthetic polymer, poly (dG-dC), can facilitate the transition from B- to Z-form DNA under physiological conditions⁹. The B-Z transition is also accompanied by changes in the torsional stress of the DNA. In prokaryotes, specific proteins, topoisomerases and DNA gyrase, precisely control the degree of supercoiling of the DNA, which in turn controls transcriptional activity. Although there is no direct evidence for torsional stress in eukaryotic DNA, it is possible that small stretches of DNA can become torsionally stressed, at least transiently. Rich and his colleagues¹⁰ have proposed that selective demethylation of Z-DNA, thereby destabilizing it, would result in torsional stress. This stress could be overcome by positive supercoiling, which would result in unwinding of the double helix at a position removed from the initial event, and yield a nuclease hypersensitive single-stranded region. There is indirect evidence for altered DNA structures, especially single-stranded regions, in the 5' region of the active human α - and β -globin genes, as demonstrated by their susceptibility to S1 nuclease cleavage¹¹. There have also been some suggestions that Z-DNA may play a positive regulatory role. On the SV40 minichromosome, the sequences that enhance transcription ('enhancers', see next section) situated upstream of the SV40 origin of replication, have the ability to adopt a Z-DNA conformation. The B-Z junction is more susceptible to nucleases and this may induce DNase I hypersensitive sites in the vicinity of the enhancer sequences.

(c) *Modifications in protein composition:* There is considerable correlative evidence for changes in the protein composition of active chromatin⁵. These modifications are chiefly in histone composition and in the non-histone high mobility group proteins (HMGs). In addition, the occurrence of sequence-specific regulatory proteins has been confirmed in some systems and these have been characterized to a limited extent, as discussed in a later section.

The chemical modifications of histones most often implicated in the regulation of transcription are poly (ADP)-ribosylation and acetylation. It is not clear whether poly (ADP)-ribosylated histones are associated only with transcriptionally active chromatin but it has been suggested that the poly (ADP)-ribosylation of histones may facilitate transcription by relaxing chromatin structure and preventing the formation of higher order configurations. Acetylation (and hyperacetylation) of histones can be correlated with active chromatin regions. It has been suggested that neutralization of the positive charge on histones by acetylation may destabilize the 30 nm chromatin fibre and facilitate its unwinding. Butyrate can inhibit histone deacetylase, resulting in histone hyperacetylation and induction of DNase I sensitivity. Thus histone hyperacetylation appears to be a permissive condition for the activation of chromatin. In addition, histones H2A, H2B and H3 have non-allelic variants with simple amino acid substitutions. These variants are conserved in evolution and have been shown to change precisely during the cell cycle of mammalian cells and during embryonic development in the chicken. Although their function is not known, it is possible that histone variants are involved in transcriptional regulation by altering the structure of nucleosomes. However, their occurrence in active chromatin has not been studied extensively.

The HMGs are highly conserved DNA-binding proteins of low molecular weight and contain ~25% basic and ~30% acidic residues¹². In studies with chicken erythrocytes and trout testes, an enrichment of HMGs has been demonstrated in active chromatin fractions and HMGs have been postulated to confer DNase I sensitivity on active genes. Antibodies against HMGs can bind preferentially to active heat shock genes in *Drosophila*. However, HMGs are associated with non-transcribed chromatin regions also. *In vitro* HMGs can unwind supercoiled plasmids, partially inhibit histone deacetylases and bind

preferentially to single-stranded DNA. Their role *in vivo* may be to maintain nucleosomes in an altered configuration in active chromatin.

(d) *Nucleosome positioning*: Unique positioning of nucleosomes at specific sequences could, in principle, regulate gene activity by controlling exposure of various sequences such as promoters, enhancers etc to enzymes and regulatory molecules. Nucleosomes could also be 'phased', that is, regularly placed on a repeating DNA sequence such as satellite DNA. On the other hand, such positioning or phasing may not be necessary, as the DNA wound around nucleosomes appears to be reasonably accessible to macromolecules. The location of nucleosomes with respect to DNA sequence depends on the enhanced cleavage by enzymes or chemicals at nucleosome-free regions of chromatin. However, since most reagents also have a certain degree of sequence specificity, there is conflicting data in the area. Most studies have concentrated on the highly repetitive α -satellite DNA in African green monkey cells, where the finding of a unique restriction site within micrococcal nuclease-cleaved chromatin subunits was interpreted to mean that the nucleosomes were in one defined location of the repeat unit of the satellite DNA⁵. However, additional data have suggested that micrococcal nuclease has preferential cutting sites on DNA. At best there may be a small number of phased nucleosomes superimposed on a largely random nucleosome positioning. There are similar uncertainties on nucleosome positioning in protein-coding genes and 5s rDNA genes. It is clear that better analytical techniques are required before this issue can be resolved.

MECHANISM OF GENE TRANSCRIPTION

The active chromatin configuration of a gene gives it the potential for being transcribed by RNA polymerase and associated factors. Eukaryotic RNA polymerases can be divided into three classes: polymerase I transcribes 28s and 18s rDNA genes, polymerase II transcribes

protein-coding genes and polymerase III transcribes 5s rDNA and tDNA genes. For polymerase III promoters, sequences in the middle of the coding regions are required for the transcription of 5s rDNA genes (see next section) whereas sequences at both the 5' and 3' ends of tDNA genes are essential for transcription¹³. The 5' ends of genes transcribed by RNA polymerase II contain several promoter-like sequences¹⁴. About 30 nucleotides upstream from the transcription initiation site is a short AT-rich nucleotide sequence often called the TATA box. Most evidence indicates that the TATA box determines the specific site of initiation of transcription *in vivo*, but not the efficiency of initiation (as initiation from aberrant sites can occur *in vivo*). However, it appears to be essential for *in vitro* transcription, as its elimination (or conversion to TAGA) drastically reduces initiation of transcription in the chicken conalbumin and ovalbumin genes and the β -globin gene. Several genes also have a CAAT sequence about 80 nucleotides from the transcription initiation site as well as GC-rich sequences flanking the CAAT sequence, both of which can modulate the rate of transcription. Proteins that specifically bind to the TATA box have been identified in *Drosophila* and HeLa cells. Specific proteins that bind to the GC and CAAT boxes have also been identified. Protein SP1 binds to the GC box at the 5' ends of several genes and enhances transcription 10–50 fold. These factors probably form a complex with promoter sequences prior to initiation and may assist in the recognition of promoter sequences by RNA polymerase II. Control of the rate of transcription initiation is one way by which the expression of genes can be varied.

Recently, sequences have been described that can enhance transcription up to 200-fold, called enhancers⁵. These were first discovered in the 5' ends of the SV40 early region genes as a 72 bp direct repeat, which was essential for transcription of the viral early genes and could enhance transcription of heterologous genes such as the cloned β -globin gene about 200-

fold. The enhancer was effective either upstream or downstream of the gene at distances of several kb and in either orientation. Enhancers have been detected in several DNA viruses and retroviruses. They apparently contain or are close to DNase I hypersensitive sites and may adopt altered DNA structures as seen in an earlier section. Putative eukaryotic enhancers have been identified in the immunoglobulin, insulin and chymotrypsin genes. These enhancers appear to be active only in the tissues which express these genes. Since the enhancer sequences themselves are present in all tissues, it is most likely that the enhancers are interacting with tissue-specific regulatory factors to enhance the transcription of specific genes. Different enhancers do not appear to have sequence homology to each other. However, cellular enhancers can enhance transcription of heterologous genes on plasmids if injected into cells containing regulatory factors specific for the particular enhancers. Recent studies on regulatory factors are discussed below.

PROTEINS THAT REGULATE SPECIFIC GENES

The information available on sequence-specific regulatory proteins in eukaryotes is rather limited. In most cases where such proteins have been detected, details of their mode of action are not available due to technical difficulties in the isolation and assay of molecules present in very low concentrations. A well-characterized regulatory protein is the TFIIIA protein (MW 37,000) from *Xenopus* oocytes that binds to the intragenic regulatory sequence of the 5s rDNA genes and appears to act as a positive control molecule in the formation of a stable 'initiation complex'. TFIIIA is also required for the reconstitution of transcriptionally active 5s rDNA chromatin *in vitro*¹⁵. Using highly sensitive techniques of detection, a protein has been identified in *Drosophila* which activates transcription from the heat shock genes by binding to a site

approximately 100 bp upstream of the transcription initiation site¹⁶. This protein is present only in heat-shocked *Drosophila* cells and not in non-induced cells. Proteins that bind selectively to the chicken β -globin gene have been isolated from chicken erythrocyte nuclei. These factors can confer a pattern of DNase I hypersensitivity in the 5' region of the cloned β -globin gene which is identical to that found *in vivo*, indicating that these factors are found on the transcriptionally active β -globin gene *in vivo*¹⁷. Two different hypersensitive regions appear to bind different components of the isolated factors. Such a complexity of interacting factors is also seen in the mating type locus of yeast, the MAT locus. In this instance, the inactive loci, termed HML, are repressed by at least four factors collectively termed SIR¹⁸. Sequence-specific regulatory proteins have been identified in various viruses⁵. A complex of proteins known to bind steroid hormones, binds to enhancer sequences in the LTR of mouse mammary tumour virus. The SV40 large T antigen binds to specific sequences in the viral origin region and represses early gene transcription (and activates replication). Analogous examples are the polyoma T antigen and the adenovirus DNA binding protein.

A diagrammatic representation of inactive and active chromatin domains of structural genes is shown in figure 1.

ACTIVATION OF THE GLOBIN GENE DURING EMBRYONIC DEVELOPMENT

During early development in most vertebrates, a fetal type of haemoglobin molecule is produced by the circulating red blood cells, which eventually switches to adult haemoglobin as development proceeds. Fetal haemoglobin is produced in 'primitive' red cells which also show other embryonic markers. This line of cells then gives rise to the adult 'definitive' line of adult red cells which produce adult haemoglobin and other adult markers. Weintraub and coworkers¹⁹ have carried out a detailed study of the activation of the fetal and adult globin genes during erythro-

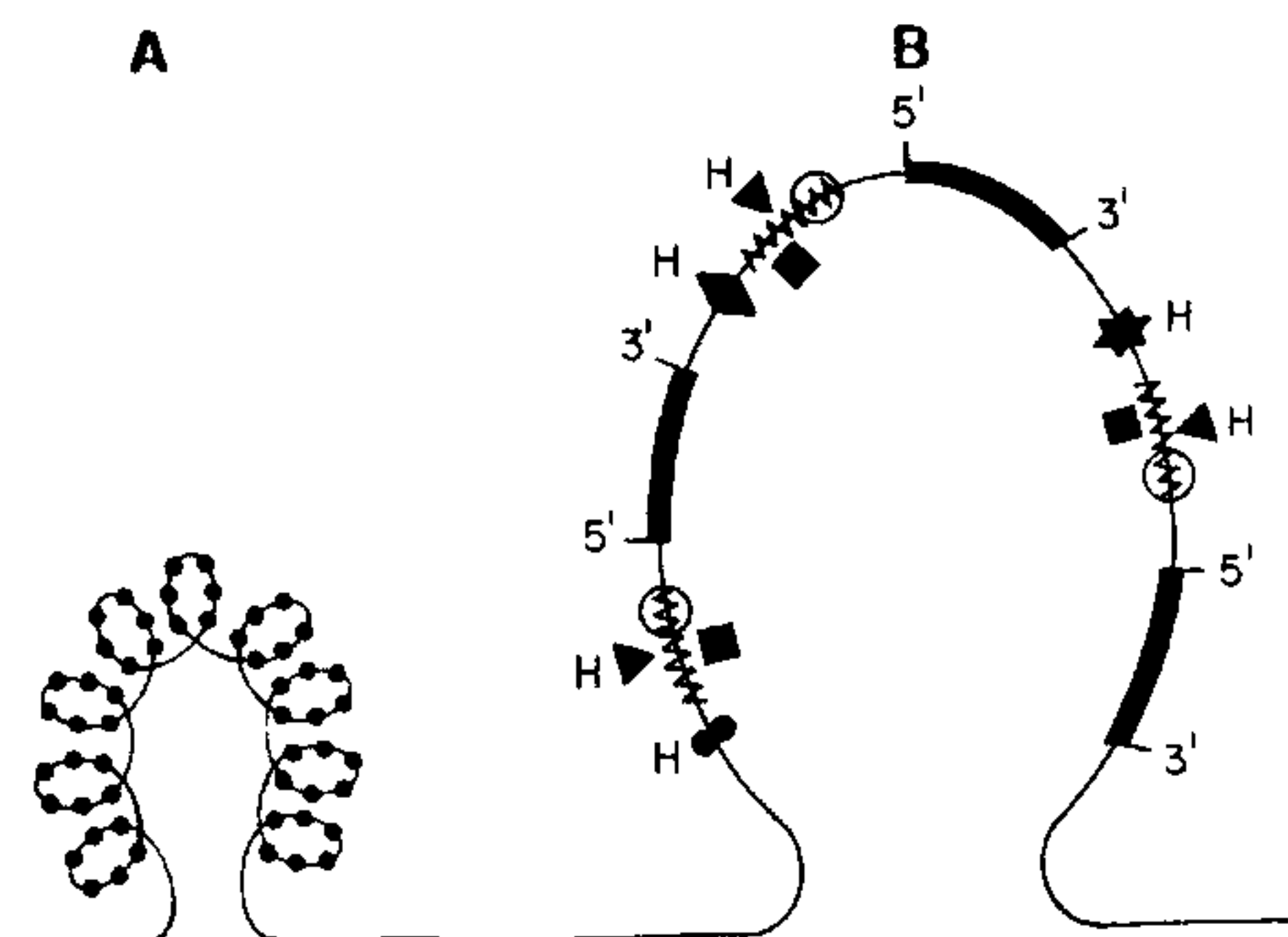


Figure 1. Diagrammatic representation of inactive and active chromatin domains. **A.** Inactive chromatin: condensed 30 nm 'solenoid' with six nucleosomes per turn. **B.** Active chromatin: relaxed chromatin domain for three different structural genes; —■— coding sequences; ~~~~~ 5' promoter region with altered DNA structure and methylation levels; ○ RNA polymerase II; ▼, ■, factors associated with RNA polymerase II; Ⓢ, ◆, ★ gene-specific regulatory factors; 'H' DNase I hypersensitive sites.

poiesis in the chicken and have been able to provide some insight into how these events may be regulated. Gene activation has been monitored by sensitivity of the gene to DNase I. The expression of the gene has been determined by assaying for globin transcripts. In fetal red cells, both adult and fetal β -globin genes are very sensitive to DNase I although only the fetal gene is transcribed. In adult lines, the fetal β -globin gene becomes relatively more resistant (and transcriptionally inactive) but the adult gene remains highly sensitive. Both genes are insensitive to DNase I in other tissues such as brain and lymphoid cells. These results imply that during the switch from the primitive to definitive line, the fetal gene chromatin is altered in some way to make it less accessible to DNase I. There is suggestive evidence that this switch is regulated by DNA sequences flanking the fetal gene, since these sequences are deleted in the disease 'pancellular hereditary persistence of fetal haemoglobin' wherein most adult red cells continue to produce fetal haemoglobin.

The above results demonstrate that the adult globin gene has already become 'committed' to activation in the fetal cells. Thus an altered chromatin configuration clearly precedes transcription of the gene. These changes in the adult gene chromatin occur very early in development. The adult and fetal gene chromatin is insensitive to DNase I in primitive red cell precursors at 20–23 hr of development but becomes sensitive by 35 hr of development i.e. after at least one round of DNA replication²⁰. Studies with inhibitors of replication indicate that replication is absolutely required for changes in chromatin structure to occur. This may reflect the necessity to restructure an active chromosome configuration, either by removing methylated DNA or by allowing different DNA binding proteins to associate with these genes. Weintraub *et al* suggest that the DNase sensitivity of the globin genes is partly a consequence of their association with HMGs 14 and 17.

An important question in the regulation of gene activation is whether the various structural changes that occur in the conversion to active chromatin are obligatorily related or independently established. To answer this requires a synchronized population of cells which can be manipulated to turn on/off a defined pattern of genes. Precursor red cells are an inappropriate choice as the transition from inactive to active globin chromatin in these cells occurs asynchronously and rapidly. Weintraub *et al*²¹ have analyzed erythroblast cell lines transformed by a temperature-sensitive avian erythroblastosis retrovirus which bears a temperature-sensitive lesion in the transforming gene *erb*. These cells are similar to normal immature red cells that are arrested in a particular stage of development. The cell line used in this particular study did not produce haemoglobin at the permissive temperature (36°C) but began to make haemoglobin at 42°C when the transforming gene was inactive. Different clones of this line were found to have acquired various changes at the globin locus. One clone had acquired DNase I hypersensi-

tive sites (at 36°C) followed by DNA undermethylation near the 5' end of the adult β -globin gene (at 42°C). However, in the same clone and another clone for the α -globin gene, undermethylation appeared to be an early marker and occurred at 36°C. For both α - and β -globin genes, binding of HMGs occurred only when the genes were transcribed (i.e. at 42°C). It appears that multiple, independent events are required for establishing the various features of fully active chromatin, which are not obligatorily linked to each other. Thus activities from different genes (regulatory) may be required for each structural change in active chromatin.

CONCLUSIONS

In this article, we have reviewed the current status on the mechanisms involved in the transcriptional activation of eukaryotic genes. Emphasis has been placed on the changes that occur initially to open up or 'activate' a specific region of chromatin, which may be considered 'gene commitment', since this would be the focal point of regulation during cell differentiation. However, it is evident that there is a certain degree of overlap between factors involved in chromatin activation and the actual transcription of the gene, such as DNase I hypersensitive sites. Recent information has been included on proteins that regulate specific genes, which is an area of intense research at present. In order to illustrate the complexity of gene regulation during embryonic development, we have discussed the regulation of the chicken embryonic and adult globin genes. The chief conclusions that emerge from these studies are that chromatin activation clearly precedes transcription of a gene; that DNA replication is essential before an active chromatin configuration can be established; and that the various changes in DNA and proteins that occur during chromatin activation appear to be independent events, not obligatorily related to each other. It is evident that these are preliminary findings and more work re-

quires to be done in this area before a meaningful model can be established. Analytical tools such as better assays for detection of small quantities of regulatory proteins and transfection of defined sets of genes into cultured cells should definitely prove to be of tremendous potential for the study of chromatin structure and gene regulation.

5 July 1986

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ANNOUNCEMENTS

EISENHOWER FELLOWSHIP

The Eisenhower Fellowship has been instituted in the honour of Dwight David Eisenhower for his contribution to humanity as a soldier, statesman and leader. The Fellowships provide each fellow twelve weeks of closely scheduled professional consultations, visits, conferences and social activities throughout the United States, during March through May 1987. The program is aimed at enhancing the professional capabilities of leaders from many nations and also fostering understanding through international fellowships.

Dr R. Sarin, Scientist and Head, Basic Research and Training Division of the National Environmental Engineering Research Institute, Nagpur has been awarded the Eisenhower Fellowship.

This is the first time that an Indian has been awarded this prestigious and distinguished fellowship, instituted by the Eisenhower Fellowship Incorporated, USA. Mrs. Shobha Sarin, wife of Dr Sarin has also been invited by the Eisenhower Exchange Fellowships Inc., to accompany Dr Sarin during his tenure; during March–May 1987.

TRAINING COURSES AT THE STRUCTURAL ENGINEERING RESEARCH CENTRE, MADRAS

The following three training courses will be conducted at the Structural Engineering Research Centre, Taramani, Madras: 1. Course on Dynamics of Civil Engineering Structures will be held during January 19–23, 1987; 2. Course on Mass-produced Precast Concrete Components will be held during January 27–30, 1987; 3. Course on Applications of Experimental Mechanics Techniques to Industrial Problems will be held during February 2–6, 1987.

The venue for all the courses is the Lecture Hall of the CSIR Campus, Taramani, Madras.

Further particulars about the above three courses may be had from Dr P. Srinivasulu, Shri V. S. Parameswaran and Dr R. Narayanan, respectively of The Structural Engineering Research Centre, CSIR Campus, Taramani, Madras 600 113.

PROFESSOR HIRA LAL CHAKRAVARTY AWARDS

Professor Hira Lal Chakravarty Awards, instituted in 1984, are intended to honour talented young scientists doing significant researches in botany within the country. There are two awards given annually, each carrying a sum of Rs. 4,000/- and a certificate. The scientists eligible for consideration of this award must have a Ph.D. degree in any branch of botany — either pure or applied. The

awards are given on original independent published work carried out in India *within three years prior to the award*. The presentation of awards to the recipients are made during the inaugural function of the Indian Science Congress. Last date of submitting application for the awards is July 15, of each year.

Further details may be had from: The Secretary, 14, Dr Biresh Guha Street, Calcutta 700 017.