STRUCTURE OF TRANSCRIPTIONALLY ACTIVE CHROMATIN

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I. INTRODUCTION

The basic unit of chromatin organization in eukaryotic cells is the nucleosome, a repeating subunit constructed by wrapping 166 to 168 bp of DNA into two left-handed superhelical turns around an octamer of histone proteins (2 molecules of H2A, H2B, H3, and H4). One molecule of histone H1 is bound to DNA sequences entering and leaving the particle stabilizing this regions. A variable length of linker DNA (few to 70 bp) is found between neighboring particles. Limited digestion with micrococcal nuclease that cleaves in between the particles gives rise to a mixture of oligonucleosomes such that the DNA length is an integer multiple of the DNA content of a single nucleosome plus the linker DNA. Further digestion with this nuclease gives rise to nucleosome core particles lacking H1 and containing only 146 to 148 bp ($1^3/_4$ turns of DNA). These core particles have been crystalized and their structure has been studied. Although the X-ray diffraction pattern is presently limited to 7 Å resolution, combined with our knowledge of histone-histone and histone-DNA interactions, they provide a good indication of the real structure of this particle.[1] It is certain that further improvement in the resolution of crystals will be required before we will be able to build a precise atomic model of the core particle.

A second level of organization concerns the packing of the linear nucleosome filament into a higher order structure. This structure can be a 30 nm solenoid that results from the folding of the nucleosomes into an helix with 6 particles per turn or perhaps a superbeaded configuration.[2-4] This higher order structure is dependent on the presence of histone H1.[5] Several recent reviews[6-9] dealt with these structural aspects of chromatin and here we will only discuss the possible relation between nucleosome structure, its assembly in higher order structures, and gene activity.

Digestion of nuclei with DNase I reveals an interesting difference between transcriptionally active and inactive genes. Active genes exhibit increased sensitivity to this nuclease relative to their inactive counterparts.[10,11] Active genes are also preferentially solubilized by brief micrococcal nuclease treatment.[12,13] These differences may be due to a less tight packaging of chromatin containing active genes relative to more compact chromatin containing inactive genes. Recent work by Weintraub for example suggests that histone H1 may be bound differently to active or inactive chromatin; it may be involved in assembly of repressed sequences into inactive supranucleosomal structures.[14] Much evidence suggests that active genes are found in modified chromatin containing highly acetylated histone, nonhistone proteins like HMG 14 and 17, ubiquitinated H2A etc., such modified structures may be more sensitive to nucleases.[15] The two hypotheses are not mutually exclusive; modification of the primary nucleosome structure of active genes may also result in a modified higher order structure.

Another property of many active genes is the presence of sites hypersensitive to DNase I or micrococcal nuclease cleavage. This property seems to be superimposed upon the generally increased sensitivity discussed above. When DNA prepared from nuclei is cleaved with a restriction enzyme, fractionated by gel electrophoresis, and analyzed by Southern blotting with a specific probe, a single fragment hybridizes to the probe if it is contained within the restriction fragment. If the same experiment is repeated with DNA isolated from nuclei that were previously treated for increasing periods with DNase I, the yield of the restriction enzyme generated band decreases. This decrease is faster for active genes than for inactive genes from the same tissue and constitutes a demonstration of the general nuclease sensitivity of active genes.[16] In addition, certain subbands hybridizing with the same probe are revealed frequently. These subbands are generated by the internal cleavage at specific sites by DNase I. The introduction of the end labeling technique greatly facilitated the mapping of hypersensitive sites.[17,18] In this technique, a short DNA fragment abutting the restriction site is used to detect the specific DNA segments generated by double strand cleavage with the DNase I at one extremity and restriction enzyme at the other. In this way, the DNase I cleavage sites can be mapped unidirectionally from the restriction site. We will not enumerate exhaustively all the published data, however, we can cite examples that illustrate the frequent occurrence of DNase I hypersensitive sites, e.g., the genes coding for the *Drosophila melanogaster* 70 kd heat shock protein,[17,19,20] rearranged genes coding for the heavy and Kappa light chains of immunoglobulins,[21-24] mouse α and β globin,[25] chicken lysosyme,[26] ovalbumin,[27] c-myc,[28] Sgs4 gene of Drosophila,[29] histone genes of Drosophila or of sea urchins,[30,31] amplified ribosomal genes in tetrahymena,[32] and SV40 and polyoma viruses.[33-38] Similarly, nuclease hypersensitive sites were mapped around promoter and upstream activating sequences (UAS) of several yeast genes.[39-41] Thus, nuclease hypersensitive sites have been identified in many different types of genes, i.e., constitutively expressed, inducable, developmentally regulated, and viral genes. A review enumerating all the published examples of DNase I hypersensitive sites will appear elsewhere.[42]

II. NUCLEASE HYPERSENSITIVE SITES

A. Mechanism of Endonuclease Cleavage of DNA and of DNA Ligand Complexes

Studies of the products generated by brief digestion of free DNA with a micrococcal nuclease or with DNase I clearly show that these enzymes have some sequence specificity. Although every phosphodiester bond is cleaved, there is certainly a two-log difference in the frequency of cleavage between the most sensitive and the most resistant bonds (see for example the analysis of DNase I and micrococcal nuclease cleavage sites in the origin proximal sequences of SV40 in Figure 1). Klug et al.[43] have studied the DNase I cleavage rate of a decanucleotide of a defined three-dimensional structure, and have shown that this rate depends on the angle between neighboring bases — DNase I cuts rapidly at the positions of high local helical twist. Previous studies with micrococcal nuclease have shown that preferential cleavage sites occur 5′ to A and T with sequences such as CTA or CATA as hot spots for cleavage.[44,45] The enzymes cleave first one of the strands. If the other strand also contains a prefered site in close proximity, a double strand cleavage will occur. Binding of proteins to DNA will frequently, partially, or totally protect the sequence against DNase I or micrococcal nuclease digestion. The degree of protection probably depends on the nature of contacts between the protein and the DNA, on the face of the helix interacting with the protein and on the degree of steric hindrance. This property of DNA protein complexes has been widely exploited in recent years to study specific protein-DNA interactions using the DNase I footprinting technique.[46] Binding of the protein can also modify the ro-

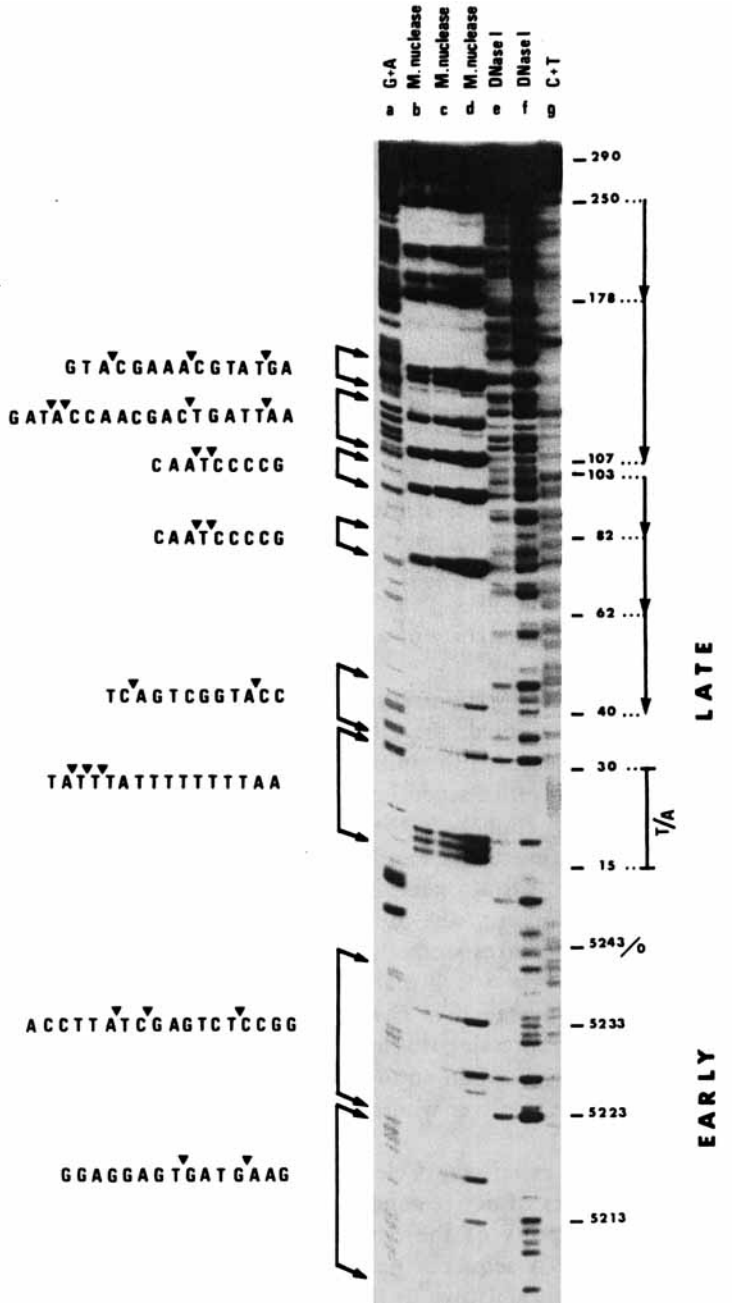

FIGURE 1. Prefered DNase I micrococcal nuclease cleavage sites in the origin-promoter-enhancer segment of SV40 DNA. The Hind III-Kpn I fragment of SV40 DNA (362 bp) labeled with p32 at the 3′ end of the Hind III site was digested with 0.3 units of micrococcal nuclease (lanes b, c, d) or 0.025 ug of DNase I (lanes e, f) at 0°C in the presence of 6 ug of nonradioactive carrier DNA. Samples were removed after 10, 20, and 30 min (b, c, and d, respectively) or 20 and 40 min (lanes e and f) and electrophoresed on a polyacrylamide sequencing gel along side chemical degradation products (A+G lane a and T+C lane g). SV40 nucleotide numbers are indicated to the right (nucleotide 5243 corresponds to the origin of DNA replication). Sequences important for early transcription (22 bp, 21 bp repeat, and the 72 bp repeat) are indicated by arrows. Some of the sequences (corresponding to the framed regions) with the micrococcal nuclease cleavage sites are indicated to the left.

tational angle of certain bases, creating hypersensitive sites on the boundary of the complex or even inside the sequence in contact with the protein. As an example, binding of *E. coli* RNA polymerase to the lac UV-5 promoter protects a region from position +17 to position −54 relative to the transcription start site at +1. Positive numbers refer to nucleotides inside the transcribed region whereas negative numbers refer to nucleotides found 5′ (or upstream) to the transcription start site. Four sites with increased frequency of cleavage relative to free DNA are detected at positions −25; −36; −37; and −48 in the coding strand. Only the last one is just at the border of the binding domain (Figure 2).[47] The cleavage sites at positions −25 and −48 are associated with frequent cuts on the complementary strand whereas the sites at positions −36, −37 are not. Hence double stranded hypersensitive cleavage sites will be observed after dissociation of the enzyme-DNA complex at positions −25 and −48 roughly (the cut on the complementary strand is staggered by 1 to 3 bp). Another system where the nuclease sensitivity of certain bonds of the double helix is greatly modified by ligand binding concerns antibiotic binding to DNA. Compounds such as actinomycin D and distamycin have a clear sequence selectivity. When they bind to DNA they greatly reduce some of the DNase I cleavage sites at the site of binding and frequently create hypersensitive sites in neighboring positions, usually on both sides of the protected sequence. The protected sequence probably results from the ligand molecules physically blocking access of the enzyme to the DNA-phosphate backbone and the hypersensitive bands from changes in the helical structure of adjacent bases.[48]

B. Location and Tissue Specificity of Hypersensitive Sites

For most of the genes studied, the DNase I hypersensitive sites are located in the sequences immediately preceding the initiation site of the transcribed RNA. However, this situation is not exclusive. In several cases, additional sites are present either inside the gene or 3′ to the gene, roughly in the region that may contain the signal for transcription termination. Furthermore, in addition to sites detected immediately upstream of the transcribed region, certain genes are preceded by discrete hypersensitive sites extending several kb upstream of the cap site. Examples are lyzozyme,[26] ovalbumin,[27] and myc.[28] In most of the cases studied, the existence of the DNase I hypersensitive sites is restricted to the tissue or cell that is expressing or is programed to express the gene in question. The hypersensitive site seen 5′ of the rat preproinsulin gene is observed in pancreatic cells expressing this gene but not in liver cells that do not synthetize insulin.[49] Similarly, the tryptophan aminotransferase gene (TAT) of the rat show several hypersensitive sites in the liver where it is synthetized but not in the kidney where it is not synthetized.[50]

These and many other experiments clearly demonstrate that the presence of hypersensitive sites is a property of active genes in a defined cellular context. Furthermore, they prove that this property of the chromosome is not just a consequence of the presence of a specific DNA sequence having a conformation that renders several diphosphate bonds extremely sensitive to DNase I. Additional control experiments consisting of the digestion of histone-free DNA also demonstrate that the hypersensitivity of certain distinct sites is not a property of protein-free DNA; enzyme cleavage sites are usually spread along the DNA sequence in this case.

C. Relation with Nucleosome-Free Region

Among the most extensively studied biological systems are the small papova viruses SV40 and polyoma. These circular double stranded genomes contain slightly more than 5000 bp. They are transcribed into two primary transcripts originating from opposite strands; the 5′ ends of the transcripts are located near the origin of DNA replication. SV40 minichromosomes harbor a nuclease-sensitive region between the origin of DNA

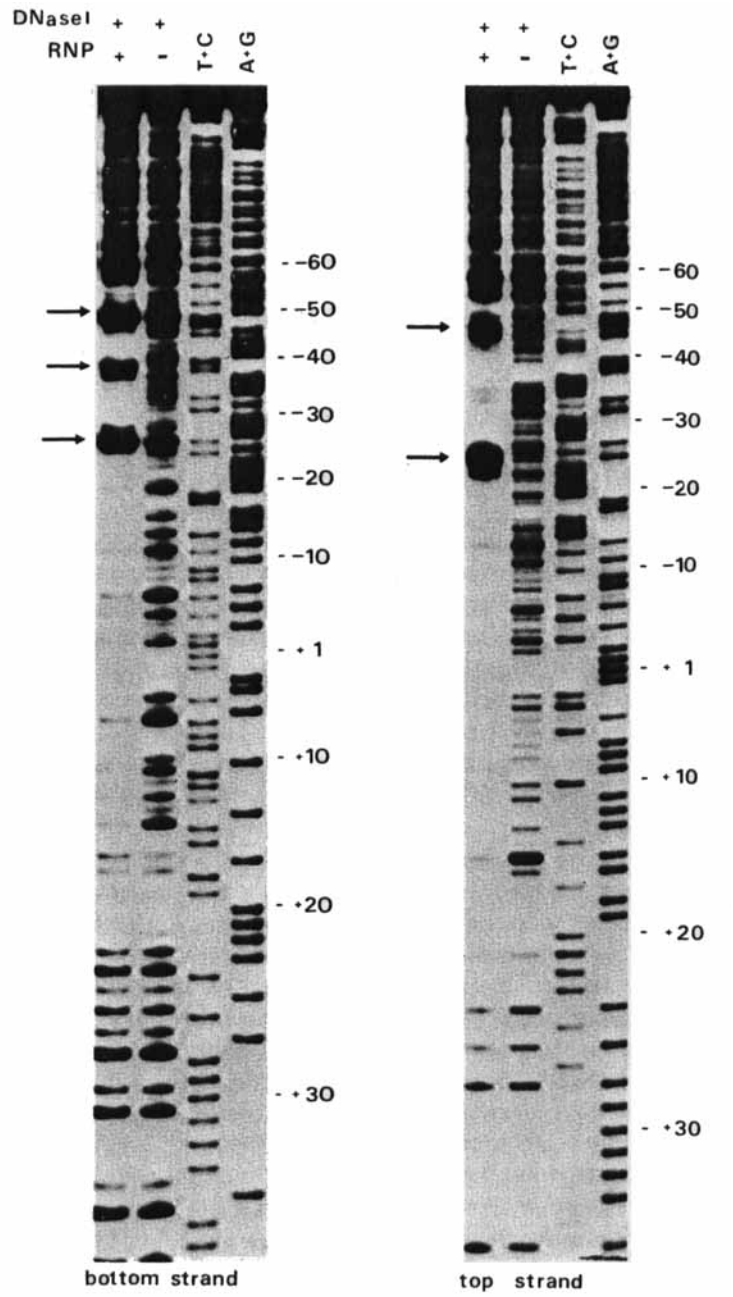

FIGURE 2. DNase I footprint of *Escherichia coli* RNA polymerase — lac UV5 promoter contacts. This modified promoter is independent of cAMP and cyclic cAMP binding protein. The top strand has the same polarity as the mRNA. The transcription initiation site is marked as +1; positive numbers designate nucleotides inside the transcribed region and negative numbers nucleotides found upstream (5′) to the initiation site. Residues +24 to −52 are protected on the top strand and residues +17 to −54 on the bottom strand. Hypersensitive sites inside the protected domain are indicated by arrows. They were mapped 5′ to nucleotides -23, -24, -45, and -47 on the top strand and -25, -36, -37, -48, and -49 on the bottom strand. T+C and A+G correspond to the chemical cleavage products.[179] (From Carpousis, A. J. and Gralla, D. J., *J. Mol. Biol,* 183, 165, 1985. With permission).

replication and the major start site of late RNA (nucleotides 1 to 325 roughly or from BglI to the HpaII restriction sites). This region is sensitive to a variety of endonucleases among which are an endogenous calcium-magnesium dependent nuclease,[33,34] DNase I,[33-36,51] and a variety of restriction endonucleases.[35] Treatment with these enzymes of either nuclei prepared from infected cells or of isolated minichromosomes result in double stranded cleavage of the viral DNA in this segment of the genome.

Electron microscopic studies of extracted SV40 minichromosomes also reveal a unique property of this region. In a fraction of the viral chromosomes (roughly 25%), a nucleosome-free segment of DNA that we defined as a "gap" was observed in the circular nucleoprotein complexes. This segment, containing roughly 400 bp was mapped by restriction analysis to a region between the origin of DNA replication and the 5′ end of the late RNA.[36,52] (The absence of histones along this region was further confirmed by its sensitivity to Bal31 exonuclease digestion following cleavage by BglI or HpaII.)[37] Thus, both biochemical tools and electron microscopic observations reveal the presence of a unique region on SV40 minichromosomes. The presence of a nucleosome-free region in a fraction of the minichromosomes was not a result of the dissociation of one or two histone octamers since the total number of nucleosomes counted in gapped or ungapped molecules was identical.[36] These experiments demonstrate an intimate association between a nuclease hypersensitive region and chromosomal DNA excluded from nucleosomes. At first, it appeared that certain DNA sequences were not packaged into nucleosomes, perhaps being protein free to interact directly with the factors required to control initiation of transcription and replication. However, one has to take into account that the positive staining technique used in the electron microscopy studies (uranyl acetate) outlines mainly the contour of the DNA and not of the proteins. Furthermore when the fine pattern of DNase I or micrococcal nuclease digestion of viral minichromosomes and of free DNA was compared, many differences appeared in this region.[53-55]

The picture that emerges is that a segment of DNA can be excluded from nucleosomes, becoming more sensitive to nuclease digestion than nucleosomal DNA and behaving like an open window in the chromosome structure. However, the sensitivity of this "open window" is modulated by certain nonhistone proteins, creating a series of protected and hypersensitive sequences. (Consistent with this notion is the identification of Z DNA binding proteins isolated from SV40 minichromosomes that appear to bind to two Z DNA forming segments in the viral enhancer.)[56] Moreover, we have evidence that at least four cellular proteins are bound to the gapped region of SV40 containing the viral enhancer and early promoter elements (Figure 3).[55] (One of them may be identical to the Z DNA binding protein isolated by Azorin and Rich.)[56] The binding of these proteins is not limited to the small fraction of minichromosomes actively engaged in transcription or replication; instead it is a property of a large fraction of the intracellular viral minichromosomes. Although previous observations suggested that only a fraction of the viral minichromosomes possess a hypersensitive region, we presently believe that most of the intracellular viral chromatin contain a nucleosome-free region (unpublished observations). A similar result is found with the chicken β-globin gene. This gene is preceded by a nuclease hypersensitive region of roughly 200 bp. In this case at least part of the presumably histone-free DNA is associated with a transcription control factor as will be discussed later.[56,57]

D. Hypersensitive Sites in Active Chromatin are Defined by Specific Nucleotide Sequences

The occurrence of nuclease hypersensitive sites may be a global property of an entire domain of chromatin. For example, specific bending or kinking of a nucleosome fiber or of a solenoid might produce a histone-free segment hypersensitive to nucleases. In

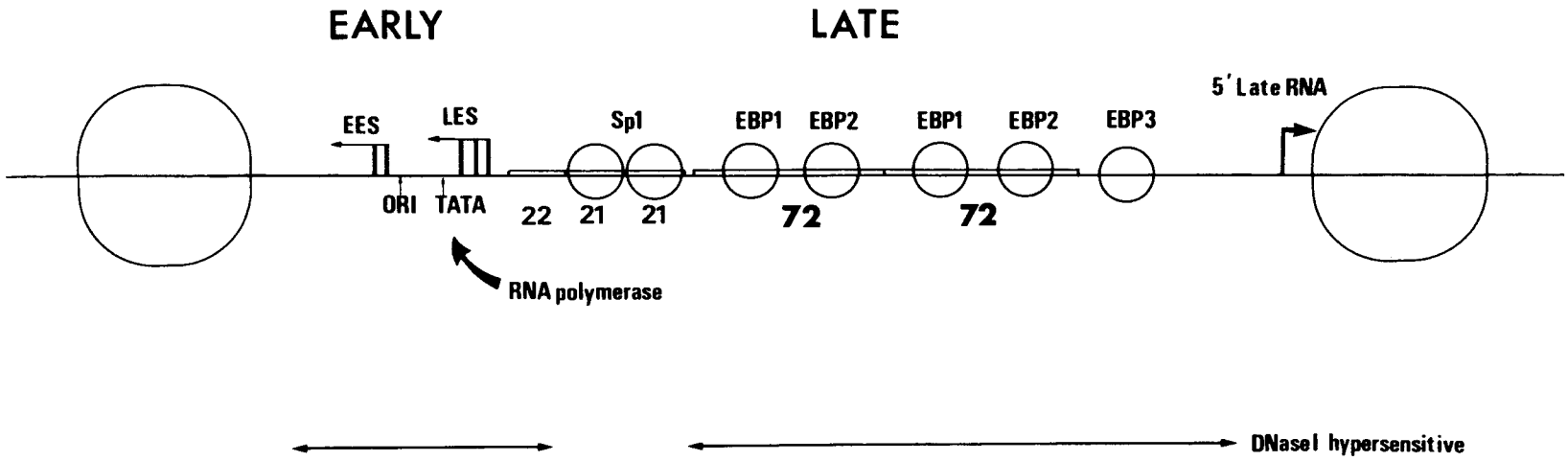

FIGURE 3. A model for the structure of the origin-promoter-enhancer region of SV40 in chromatin. The initiation sites for early RNA (EES early early start sites and LES late early start sites) and for late RNA, the functional elements of the early promoter including TATA sequence, the 21 bp and the 72 bp repeats are indicated. Micrococcal nuclease footprinting in nuclei followed by genomic sequencing analysis of both strands suggests that the 21 bp repeats are protected by the Sp1 transcription factor[180] and that three different enhancer binding proteins (EBP1, EBP2, and EBP3 covering the SphI, EcoRII, and PvuII restriction sites, respectively) are associated with nucleotide sequences important for enhancer function in the 72 bp repeats and on its late side.[55] Both Sp1 and the enhancer binding proteins are easily removed from the viral mini-chromosomes by low salt wash. Also indicated the DNase I hypersensitive region previously mapped by the analysis of double stranded cleavage. This sensitive region is interrupted in the 21 bp repeats. Nucleosomes (large circles) were placed on the borders of the DNase I hypersensitive region.

this case, the sequences that define the enzyme sensitive segment would be embedded in nucleosomal structures; the DNA sequences present in the hypersensitive site would be somewhat neutral. Alternatively, the generation of a window in the chromatin structure might be a property of a specific nucleotide sequence, which on association with nonhistone proteins would be excluded from a nucleosomal structure and would generate nuclease hypersensitive sites. To try to distinguish between these possibilities, the chromatin structure of chimeric recombinant viruses or plasmids, or of naturally occurring or artificially created mutants was studied. With SV40, recombinant viruses were generated where the DNA sequences corresponding to the nuclease sensitive segment were duplicated by their insertion on the opposite side of the genome between the early and late polyadenylation sites.[51,59] These transposed sequences were also hypersensitive to DNase I, thus the chimeric virus created contained two hypersensitive segments. A recent electron microscopic study has shown that both the original and the copy of the nuclease sensitive DNA sequences can be seen as nucleosome-free gap; some molecules even contain two gaps.[51] Moreover, when plasmids containing only a small fragment of SV40 DNA were introduced into cos cells (a monkey cell line that synthetize constitutively the SV40 T antigen and replicate any exogenous DNA harboring the viral origin of replication)[60] a typical DNase I hypersensitive pattern was observed between the BglI and the HpaII sites after replication.[54,61]

Newly replicated SV40 minichromosomes do not seem to harbor a hypersensitive region along the origin promoter enhancer sequences.[62,63] It is possible in this case that these sequences became associated with specific proteins only some time after the completion of replication. This association can be coupled to a rearrangement of nucleosomes around the viral DNA or to the loss of one or two nucleosomes.

Beckendorf et al.[29,64] analyzed the structure and expression of the wild type and variant Drosophila glue protein gene Sgs4. These variants contain small deletions or sequence changes in the region −300 to −500 bp upstream of the transcription start site, some of which reduce or abolish transcription. The modifications in the DNase I hypersensitivity pattern observed for the wild type strain correlate with the effect on transcription, mutants that reduce transcription affect these sites. In contrast, several parts of the sequence surrounding this region can be changed without affecting transcription or DNase I hypersensitivity.

These experiments clearly indicate that hypersensitivity is defined by a relatively short nucleotide sequence and does not require the integrity of the viral chromosome or a domain of cellular chromatin. However, as already mentioned, this sequence-dependent hypersensitivity is observed only in tissues that express or are programmed to express the gene.

Sequences that are hypersensitive to DNase I are usually included in the undermethylated domains of active genes.[65] It was suggested that hypomethylation during spermatogenesis of sequences that control the expression of house-keeping genes can be maintained along development.[66] Both the local undermethylation and the DNase I hypersensitivity can be related to the presence of specific proteins at these sites as we will discuss below.

E. Regulatory Functions of DNase I Hypersensitive Sequences

Many of the DNase I hypersensitive sites are observed in sequences preceding the start site of transcription of a gene in tissues that express such a gene. It is reasonable to postulate that these sites are intimately associated with gene activity, they may arise as a consequence of the transcription process or alternatively they may play a crucial role permitting this transcription. The acquisition of at least some of the hypersensitive sites is a prerequisite for transcription activation and not a consequence of the process of transcription. In a number of systems DNase I sites appear during development

before transcription actually starts and they are maintained after transcription ceases.[67,68]

The studies of transcription control sequences in eukaryotes have revealed several important elements; the cap proximal sequences include the TATA box or Hogness-Goldberg box found roughly 30 bp preceding the start site of transcription. Another consensus sequence, the CAAT box, is found frequently between positions −70 to −80.[69] For several promoters, e. g., herpes simplex 1 tK or β-globin,[70,71] sequences up to roughly position −110 are required for efficient activity in vivo. These sequences contain two GC rich tracts in the case of tK or a nonanucleotide common to several adult β-globin genes as additional elements crucial for promoter activity. Other cellular promoters may require additional upstream sequences, e.g., rat or human insulin.[72] In many cases the upstream promoter control sequences required for gene expression are contained within the 5′ proximal hypersensitive site (hsp 70,[17,19,20] rat insulin,[49] β-globin, tK,[73,74] etc.).

The control sequences are certainly more complex for the early promoters of viruses like SV40 or polyoma. It was shown that sequences that are found between −110 and −275 relative to the start site of viral early RNA are required for the full activity of early promoter in SV40.[75-77] Similarly, for the polyoma early promoter sequences that are found between −180 and −410 relative to the cap site are crucial for promoter activity.[78] Construction of chimeric plasmids or viruses have shown that these far upstream control sequences have an additional property. They can enhance the transcription from a promoter when placed in cis either 5′ or 3′ and in both possible orientation to a transcription unit.[79-81] The DNA sequences, having this cis enhancing function, that were defined as enhancers are included in the DNase I hypersensitive segment of both SV40 and polyoma.[36,38,82] Fromm and Berg[81] have shown in addition that displacement of the 72 bp repeat of SV40 — the enhancer element — inside the early transcription unit or 3′ to it in a virus originally lacking these sequences restores the viability of the virus in three out of the four possible configurations. In the last configuration, the 72 bp repeat is incapable of restoring viral viability, perhaps due to interference of neighboring viral sequences with the enhancer function. When scored for DNase I hypersensitivity, the three active constructions create a hypersensitive site in the enhancer sequences whereas the inactive construction lacks such a site. These results clearly show that enhancer function is intimately associated with DNase I hypersensitivity.

Another example that extends this correlation comes from studies on polyoma virus. The enhancer region of polyoma, originally defined between −180 and −410 relative to the cap site, consists of at least two distinct enhancer elements.[82-87] The major one (A) contains a core sequence (GCAGGAGG) found also in the enhancer preceding the Ela gene of Adenovirus 2 and in the enhancer found between the variable and constant domain of the rearranged heavy chain gene of the mouse. The second (B) contains the core sequence found in the enhancer of SV40 (GTGGTTTTG). The entire region containing both enhancers is sensitive to DNase I. Two hypersensitive sites (HS1 and HS2) were mapped next to either one of the core sequences. Duplication of 30 nucleotides containing the Ela-like core sequence generates a new HS site.[88] In contrast, duplication of the sequences containing the hypersensitive site in enhancer B, but not the adjacent SV40 like core sequence, does not generate a new hypersensitive site.

Another example of a close association between a DNase I hypersensitive site and a functional enhancer is that of the heavy and light chains of mouse immunoglobulin. DNase I hypersensitive sites already mentioned are located for both these genes between the variable and constant domains of the somaticly rearranged gene. The same sequences were shown to contain a lymphocyte specific transcription enhancer element.[89-92]

Similarly, recent studies from Beckendorf[93] have identified an enhancer-like element in the upstream sequences (−840 to −107) of Sgs4 glue gene which coincides with the DNase I hypersensitive region mentioned above.

Finally, DNase I hypersensitive sites are also characteristic of regions not directly involved with transcription. We will discuss later the nuclease hypersensitivity of the yeast centromer sequences and of replication origins. Furthermore, a region of targeted recombination in the yeast mating type system is hypersensitive to DNase I.[39]

F. Proteins Associated with DNase I Hypersensitive Sequences

We have already mentioned that certain proteins are associated with the nucleosome free-nuclease hypersensitive region found upstream of the transcription initiation sites of SV40 or of chicken β-globin genes. In the following paragraphs we will try to summarize some of the recent evidence concerning the nature of the proteins that are involved in the creation of such DNase I hypersensitive sites.

1. Heat Shock

In a very elegant study, Wu[94,95] analyzed the structure of the nuclease hypersensitive region present near the 5′ end of the *Drosophila* hsp70 and hsp83 genes. For the hsp70 genes this region extends from position +100 to −215 with an interruption of a relatively resistant segment of 30 bp from −8 to −38. By exonuclease III digestion *in situ* of nuclei previously treated with a restriction enzyme that cleaves within the DNase I sensitive region he mapped the barriers for the exonucleolytic attack. He showed that the region between nucleotides −8 to −38 contains a protein (s) bound to sequences including and surrounding the TATA box. Upon heat shock a second barrier for exonuclease III progression is detected by bidirectional mapping and can be positioned between −40 and −108. Furthermore, proteins extracted from heat-shocked nuclei restore the protection of the second element when added to nonshocked nuclei. Wu suggests that a nucleosome-free region is present between positions +100 and −215. In normal cells the TATA box region is occupied by a specific TATA binding protein (TAB). Upon heat shock, a positive transcription regulatory factor HAP (heat shock activator protein)[96] binds upstream of the TATA box to sequences containing the consensus element found upstream of most heat shock genes.[97] The combination of both factors will stimulate initiation by RNA polymerase II (see Figure 4). The formation of the nucleosome-free segments may be generated by the binding of the TAB protein, thus facilitating the interaction between the positive activator and the upstream control sequences during the thermal treatment. However it is somehow difficult to envision how occupying only 30 bp with a protein excludes 300 bp of DNA fron nucleosomes. Perhaps the positions of the nucleosomes on both sides of the control region is defined by specific DNA sequences that phase nucleosomes, or by proteins analogous to the α-satellite binding protein that defines the location of these nucleosomes.[98] Alternatively, the entire region may be occupied by proteins even though their presence is neither detected by the exonuclease III test nor by protection of DNA against DNase I or restriction endonuclease digestion.

2. β-Globin

Another system where a nuclease sensitive region can be related to the presence of a specific factor is the chicken adult β-globin gene. As already mentioned, a region extending from roughly −60 to −260 relative to the transcription start site is exposed to DNase, micrococcal nuclease, and restriction enzymes.[57] At least part of this segment can be excised from the nuclei as a DNA-protein complex. When partially purified extracts from chicken erythrocytes are present during nucleosome assembly onto plasmids containing the β-globin gene, a nuclease sensitive region can be observed.[58]

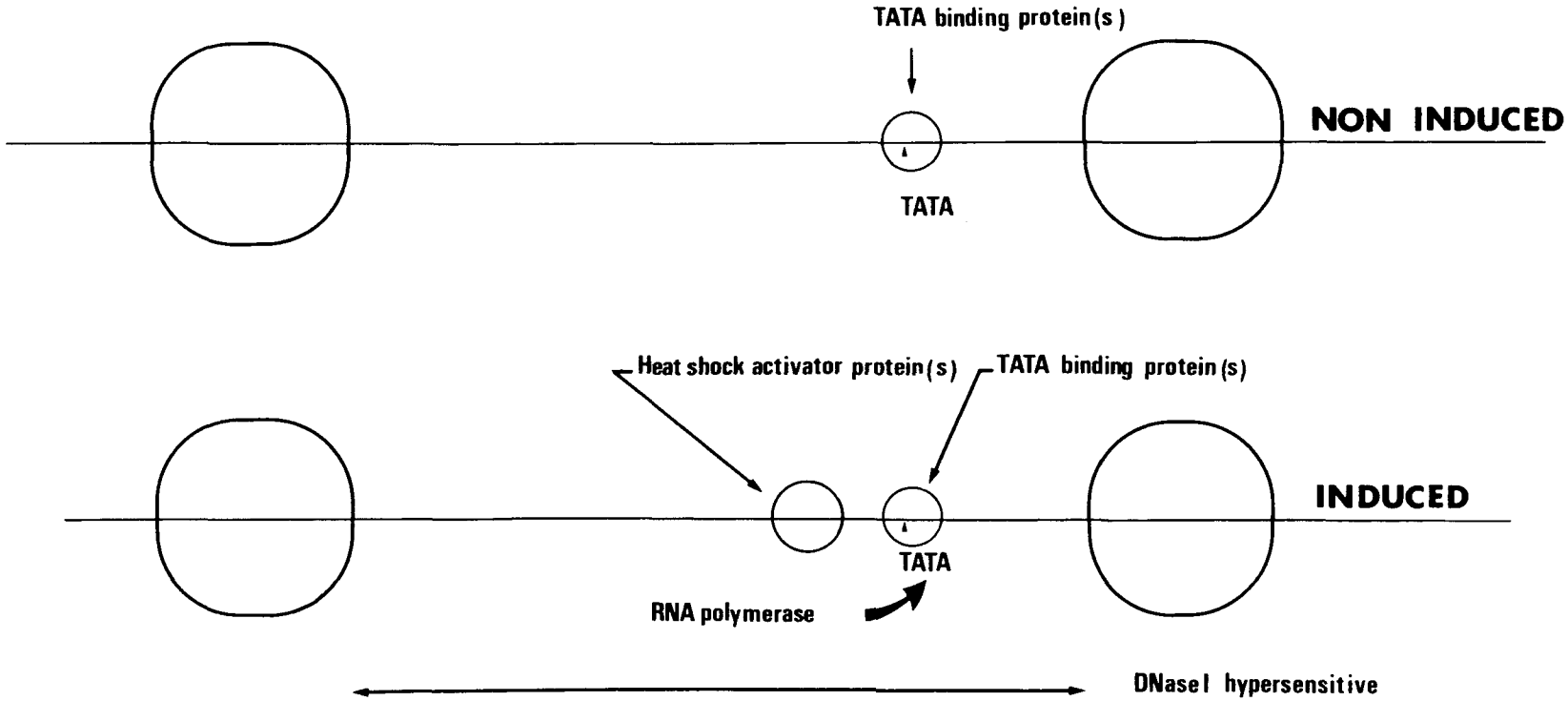

FIGURE 4. A model for the heat shock promoter region in chromatin during normal and induced states. Large circles represent nucleosomes; small circles transcription control factors. The position of nucleosomes have not been determined precisely. The positions of the TATA binding protein (TAB) and the heat shock activator protein (HAP) were deduced by exonuclease III digestion studies. (From Wu, C., *Nature (London)*, 309, 229, 1984a; 311, 81, 1984b. With permission).

3. Glucocorticoid Receptor

The expression of mouse mammary tumor virus (MMTV) is regulated by glucocorticoid hormones. The binding of the hormone receptor complex to the promoter upstream sequences, the glucocorticoid responsive element (GRE)[99] increases the rate of transcription from this promoter. The GRE behaves like a transcriptional enhancer element that is active only in the presence of bound receptor. By DNase I protection experiments three receptor binding sites were mapped between positions −72 and −305 relative to the transcription start site.[100,101] Analysis of the DNase I sensitivity of cell lines harboring a chimeric transcription unit in which the viral LTR drives the herpes tK gene in a hormone dependent manner revealed the presence of a DNase I hypersensitive site located at about −70 to −190 which coincides with the receptor binding region mapped in vitro and with the GRE enhancer localized in vivo. This site is absent in cells grown in the absence of hormone. Upon addition of the hormone the DNase I hypersensitive site is induced rapidly; it can be detected within 7 min. The same time scale required for the dectection of the increased rate of transcription from the MMTV promoter. The formation of the hypersensitive site is not a consequence of the transcription event. This site is also detected in the 3′ LTR, probably not functioning as a promoter. In addition to the hormone induced site, the integrated LTR of the virus exhibits another hypersensitive site further upstream at −860, a constitutive site that is not dependent on the hormone treatment. The addition of the hormone also increases by 4- to 10-fold the overall sensitivity to DNase I of the chromatin domain containing the chimeric viral insert. This increased global sensitivity is maintained for at least several generations after hormone withdrawal even though the hormone-dependent hypersensitive site disappeared.[102]

4. TGGCA Binding Protein

Sippel's group identified a double stranded DNA binding protein with affinity for specific sites in the flanking regions of the chicken lysosyme gene. DNase I footprint experiments revealed that the binding site contained a sequence of diad symmetry 5′ TGGCANNNTGCCA. The protein was named the TGGCA binding protein. Three binding sites were mapped upstream of the lysozyme transcription initiation sites. One of them coincides with a DNase I hypersensitive site.[26,103,104] The DNA sequences containing this site behave as a tissue specific enhancer element.[105] Further TGGCA protein binding sites were mapped in the enhancer element of BK human papova virus and in the LTR of mouse mammary tumor virus.[106] The TGGCA binding protein is probably identical to the host factor 1 required for the replication in vitro of adenovirus DNA.[106-108]

Five DNase I hypersensitive sites were mapped upstream of the human c-myc gene centered around positions −72, −289, −911, −1542, and −2012 roughly relative to the transcription initiation site of the shorter myc RNA.[28] The first site precedes this point by roughly 70 nucleotides. The second site precedes the second upstream promoter by about 120 bp. The three other sites are distributed along the far upstream noncoding region. Two of these sites (−911 and −1542) are adjacent to a sequence that interacts with the TGGCA binding protein. The center of the hypersensitive site was mapped 40 to 50 bp 5′ to the protein binding site.

G. RNA Polymerase I Transcribed Genes — Hypersensitive Sites in the Tetrahymema R-Chromatin

DNase I hypersensitive sites are not restricted to protein coding genes. The rDNA of Tetrahymena is organized as extrachromosomal, highly amplified palindromic units, each containing two genes coding for 35s pre-rRNA. The promoters are separated by a central spacer probably containing an origin of replication for the extrachromosomal

DNA. Analysis of the chromatin structure of these minichromosomes revealed the presence of DNase I hypersensitive sites upstream of the transcription initiation sites and around the transcription termination sites.[32,109] A more comprehensive study using several endonucleases (DNase I, DNase II, and micrococcal nuclease) permitted the elaboration of a scheme for nucleosome position along the nontranscribed region.[110] Three nuclease sensitive nucleosome-free regions were mapped, one immediately upstream from the 5′ end of the RNA (0.1Kb), another 0.6Kb 5′ of the RNA, and a third in the region that contains the origin of DNA replication. In the scheme proposed, one can suppose that histones are excluded from the nuclease sensitive regions by one of the mechanisms discussed above. Recent work by Westergaard et al.[111] identifies one of the proteins associated with the nuclease sensitive sites in this system. Firstly, they showed that treatment of the rDNA chromatin with sodium dodecyl sulfate followed by S1 nuclease treatment of the deproteinized DNA resulted in double stranded cleavage at the DNase I hypersensitive sites.[112] Such a treatment induces double stranded cleavage in DNA sequences bound by topoisomerase I. The cleavage *in situ* occurs at a specific nucleotide within a conserved sequence element found in two or three direct repeats at the DNase I hypersensitive sites. Furthermore partially purified topoisomerase I from Tetrahymena or pure calf thymus topoisomerase I, when added to naked rDNA, reproduce the same cleavage sites in vitro. We can conclude that topoisomerase I is bound to these sequences in vivo. The binding of the enzyme alone, or of it and other nonhistone proteins, excludes nucleosome deposition from certain DNA sequences and generate DNase I hypersensitive sites.

It is interesting to note that the nuclease sensitivity observed in this system is not dependent on gene activity, almost no difference was observed between exponentially growing and stationary cells.[110] No rRNA is synthesized in the last case.

H. Other Functional Elements — Yeast Centromers

Another clear example of functional DNase I hypersensitive sites probably not related to transcription concerns the chromosomal structure of the yeast centromer. DNase I and micrococcal nuclease digestion of yeast nuclei were used to map the chromatin fine structure around the centromers of chromosomes 3 and 2.[113,114] A discrete region of 220 to 250 bp is protected against nuclease digestion including the 130 bp that exhibit the highest degree of homology between the two centromers. This region is bounded on both sides with DNase I or micrococcal nuclease hypersensitive sites. Deproteinized DNA or salt-washed nuclei exhibit a different sensitivity pattern; the previously protected region becomes sensitive and the hypersensitive sites disappear. The same profile of protected and hypersensitive sequences is conserved when the centromer fragments are cloned into a plasmid. The nuclease resistant sequence may be in fact a microtubule attachment site or a primitive kinetochore. This sequence may bind, for example, a microtubule associated protein creating a protected region and hypersensitive sites on the edge of the protein DNA complex.

In the centromeric region, the nucleosomes found on both sides of the protected region are rather well phased relative to the DNA primary sequence; a clearly defined ladder is obtained after a secondary cleavage by a restriction enzyme of the DNA prepared from micrococcal nuclease treated nuclei. However, this phasing of nucleosomes is dependent upon the underlying DNA sequence and is not the direct consequence of the unique structure at the centromer.

I. Commitment Proteins

As we suggested above, formation of nucleosome-free nuclease hypersensitive segments of genomic DNA is dependent on the binding of certain nonhistone proteins. Such proteins bind to these sequences in some cell types but not in others since DNase

I hypersensitive sites are observed mostly in the tissue that expresses or is committed to express the gene examined. These proteins can not be associated with such DNA sequences already in the very early stages of the embryonic life, since a unique precursor stem cell can give rise to different cell lineage. A chain of events during the commitment of cells to a certain lineage should lead to the binding of such proteins and to the formation of irregularities in the nucleosome fiber structure. Perhaps following replication newly synthesized nonhistone proteins bind to certain regulatory signals on the newly replicated DNA, thus excluding formation of new nucleosomes. We propose to name such proteins *commitment proteins*. Their binding to promoter or enhancer sequences of a gene would create open windows in the chromatin that would permit the interaction of these sequences with positive transcription factors and with the RNA polymerase. As will be discussed later, modification of chromatin structure can precede the onset of transcription. According to this model, at least one of the transcription factors in question must be tissue specific. Its presence in an active form in the cell will switch on the transcription process. It can appear either concomitantly with the creation of the specific structure at the chromatin level or one or several divisions later.

In vitro systems that attempt to reproduce cell specific transcription with free DNA may depend on specific and nonspecific transcription factors alone if the role of the commitment proteins is restricted to the formation of an accessible template. However, it is perhaps more reasonable to postulate that optimal activity of these factors will require interaction with the commitment proteins. The interaction of these two classes of proteins may create stable transcription initiation complexes discussed recently by Brown.[115]

III. NUCLEOSOME ARRAY AND ACTIVE GENES

Until now we focused our discussion on alterations in the chromatin structure along the promoter or enhancer sequences of active genes. However, the entire configuration of an active gene differs from that of an inactive gene as revealed by DNase I sensitivity.[10,11] Furthermore, this general sensitivity is not limited to the coding sequences; partial nuclease sensitivity can extend several Kb on both sides of the transcription unit.[16,116-118] This sensitive chromatin configuration is not solely a consequence of the transcription event. Chick adult globin genes maintain their global sensitivity and the specific hypersensitive sites to DNase I in mature erythrocytes even though their transcriptional activity has ceased.[119] Thus, one has to speak about a chromatin domain, perhaps a loop, that has acquired structural modification that precede, in time, the active transcription of a gene and are maintained after this transcription has ceased.

Preparative separation of active and inactive chromatin is needed in order to study and understand the biochemical basis for the differences between these two entities. This fractionation is currently done after micrococcal nuclease digestion of the nuclei and is based on differential solubility of the released mono or oligo nucleosomes.[13,120-124] Another approach is to separate mononucleosome particles according to their protein composition and DNA length on two dimensional gels and to analyze the abundance of different DNA sequences in these particles.[125] Employing such techniques followed by biochemical analysis of the chromatin gave rise to a number of interesting observations. It is generally but not unanimously agreed that nucleosomes along active genes are complexed with HMG14 and HMG17, are low in histone H1, and are enriched in acetylated and ubiquinated histones.[15,122,126-131] Furthermore, they may be partially deficient in H2a and H2b and contain other nonhistone proteins like LP30 and LP32 found in active r-chromatin of *physarum*.[132,133] Inactive or repressed genes may be more abundant than active genes in mononucleosomes particles containing the H1° subspecies of the H1 family. The gene coding for α-fetoprotein which is

silent in adult liver is enriched in these particles prepared from this tissue whereas the active albumin gene sequences are under represented in the same particles.[134] However, difficulties to exclude histone H1 exchange,[135] only partial enrichment in active genes during preparation of active chromatin, and artifacts in hybridization studies that distinguish active from inactive genes complicate the interpretation of these data.[136,137]

Ciliated protozoa provide an interesting way to study active and inactive chromatin. They contain two types of functionally and morphologically distinct nuclei; the transcriptionally active macronucleus and the transcriptionally inactive micronucleus. Butler et al.[138] showed for example that the nucleosomal repeat is longer in macronuclei than in micronuclei (220 bp vs. 165 to 185 bp). Furthermore, macronuclear chromatin shows a greatly increased thermal premelt and different circular dichroism spectra.

A. Electron Microscopic Studies

Electron microscopic studies of transcriptionally active chromatin have shown that genes transcribed at a moderate rate contain nucleosome-like particles.[139] Moreover, by the use of antihistone antibodies the presence of histones in the particles observed in between the nascent RNA chains was confirmed. The situation is more complex for abundantly transcribed genes and for DNA sequences encoding ribosomal RNA. In these cases the density of nascent RNA chains is very high, so it is not easy to observe nucleosomes along these very actively transcribed genes.[140] Nevertheless, the following observations have been made. The actively transcribed sequences coding for the Balbiani Ring 2 75 S RNA of *chironomus* contain a low number of nucleosome-like particles between the nascent RNP particles. This segment of active chromatin is less compact than inactive chromatin in the same spreads. The active chromatin fiber itself has a diameter of 5 nm which is still thicker than free DNA. Daneholt et al.[141,142] suggest that the 10 nm nucleosomal fiber is modified upon transcription to form an extended, nonbeaded configuration. In other studies, an inverse correlation was observed between the number of nascent RNP chains in active genes and the compaction of the chromatin fiber.[139,143]

In a series of recent studies, Koller et al.[144-146] suggest, on the basis of electron microscopic studies, psoralen cross linking and nuclease digestion studies that actively transcribed ribosomal chromatin of *Xenopus laevis* oocytes or of *Dictyostelium discoideum* are totally devoided of nucleosomes and behave like free DNA. The psoralen cross linking followed by DNA spreading under denaturing conditions clearly shows that the nontranscribed spacer is organized in regularly spaced, single-stranded bubbles of 200 to 400 bp indicating the presence of nucleosomes, whereas the transcribed region is heavily cross linked. However, this cannot be concluded to be naked DNA, it is still possible that highly modified nucleosomes in an extended configuration will permit extensive psoralen cross linking.

B. Biochemical Studies

Biochemically, the evidence for assembly of DNA sequences in nucleosomes is the formation of a multimeric ladder of the DNA extracted from nuclei digested with micrococcal nuclease. The total DNA fractionated by electrophoresis on an agarose gel can be transferred to nitrocellulose filters and hybridized with nick translated specific probes. The appearance of the typical micrococcal ladder identical to the pattern seen for total DNA by ethidium bromide staining confirms that a specific gene from which the probe was isolated is found in regulatory spaced nucleosomes. This is the case for the hsp 70 genes of Drosophila,[147-149] for chicken ovalbumin gene in erythrocytes.[117] for the immunoglobulin Kappa light chain gene in non B cells,[124] and for many other genes studied. However, this uniform ladder disappears for the hsp 70 genes

upon heat shock, for the obalbumin gene in the oviduct, the site where this gene is actively transcribed, and for the C domain of the Kappa light chain in B lymphocyte producing Kappa chains (references cited above). Instead of a regular repeat pattern of DNA, only monomeric or dimeric bands appear in the soluble fraction and a continuum of fragments (a smear) is detected on the blot of the total DNA extracted from digested nuclei after hybridization with the specific probe.[124] Since the coding sequences can be isolated in particles that sediment or migrate like mononucleosomes[150] and DNA fragments of mono and dinucleosome length can be detected in blots,[15] it is likely that actively transcribed genes remain associated with the core histones. Nevertheless, not all studies agree with this conclusion. Karpov et al.[149] failed to detect DNA protein particles with active hsp 70 gene after DNA-protein cross linking, and Koller et al.[146] failed to detect the presence of histones on active ribosomal gene of *Dictostelium* after psoralen cross linking as already mentioned.

Three hypothesis can be formulated to explain the absence of an ordered nucleosomal repeat from highly active genes even though they are associated with histones. First, in the absence of histone H1, the structure of the nucleosome filament is not uniform but contains variable distance between neighboring particles. The micrococcal digestion of this material will generate monoparticles, closely packed diparticles and higher homologues. However, the variability in linker length will generate a continuum of DNA fragments rather than distinct bands. Second, nucleosomes along active genes have a modified configuration[133] (see definition of "lexosomes") exposing intranucleosomal DNA to micrococcal nuclease.[152] The preference for cleavage in the linker DNA is lost resulting in generation of a continuum of fragments. Third, transcription is a dynamic process involving transient short-lived dissociation of core histones to permit the propagation of the RNA polymerase. This last hypothesis is unlikely because the results discussed below clearly show that the modification of chromatin structure characteristic of active genes can already be observed in committed cells in the absence of transcription unless we postulate that this dynamic process is already set up in committed chromatin.

A recent study by Rose and Garrard[124] on the state of the Kappa and lambda light chain genes in B cells and non B cells is a good demonstration of this point. In pre B cells where the gene is not rearranged the normal micrococcal repeat is observed for the sequences coding for the constant domain of the Kappa chain. In plasmacytoma cells that synthesize this protein very actively, no clear repeat ladder is seen after nuclease digestion and analysis of DNA. After separation of the chromatin into soluble material, EDTA soluble material and insoluble material, the authors showed that up to 30% of the Kappa constant gene DNA can be found in the soluble fraction migrating as monomeric and dimeric fragments. (In fact, the dimer length was shorter than the normal repeat indicating two closely packed nucleosomes). The majority of the Kappa constant domain specific material was found in the insoluble fraction. Analysis of the specific DNA in this fraction showed a continuum of fragments and not discrete bands.

The disruption of the normal nucleosomal repeat of the Kappa coding sequences is not dependent on the active transcription of this gene. A cell line carrying a deletion in the promoter of this gene that prevents transcription still maintains the disrupted nucleosomal structure. Furthermore, the sequences coding for the lambda light chain that are not expressed in the cell line studied differ in their fractionation properties and in the distribution of micrococcal nuclease digest from an inactive gene-like globin. They seem to have an intermediate behavior between the globin and the Kappa light chain sequences. Quite similar results were obtained by Cohen and Sheffery[151] for the globin genes in mouse erythroleukemia cells. Before induction of globin synthesis, the globin genes are enriched in the insoluble chromatin fraction obtained after solubilization in

EDTA of the micrococcal nuclease digest, and they are organized into structures that are largely devoid of normal nucleosomal repeat. Following chemical induction and onset of globin transcription, globin gene enrichment and nucleosome disruption in the insoluble chromatin fraction increases. The disrupted domain includes the transcribed DNA sequences and some sequences to its 5′ end (0.3 kb for the B-major globin and 2 kb for the α1 globin). Genes not transcribed in these cells like albumin or immunoglobulins are organized into ordered nucleosomes as revealed by micrococcal nuclease generated normal repeats. House keeping genes like tubulin that are transcribed in a much lower rate than globin show a canonial array of nucleosomes and fractionates similarly to bulk DNA.

These studies reinforce the notion that the biochemical composition of a chromatin fragment that contains an active gene or a potentially active gene differs from that of a silent gene. What are the signals that govern the turning on of a process that will convert inactive chromatin into its active counterpart during differentiation of the B lymphocytes for example? Are they related to the formation of DNase I hypersensitive sites? It is possible that DNase I hypersensitive sites, present in proximity of the transcription unit or even several Kb 5′ or 3′ to it, supply this function. These can either be the same sites that are required for the control of transcription or sites that are more remote or both. Enhancer elements may also play a role in the organization of active chromatin domains even though the evidence for this role is not conclusive. These elements activate transcription of DNA introduced into cells by transfection or injection, perhaps even before the assembly of cellular histones with the incoming DNA.[153] Furthermore, the SV40 72 bp repeat activates transcription in vitro when placed in the proximity of a promoter also in the absence of chromatin assembly.[154] However, these observations do not exclude the idea that these elements also participate in the creation of an active chromatin domain in the cell. Nevertheless, once the state of active transcription was established during the B cell differentiation, e.g., the continued transcription of the endogenous immoglobulin heavy chain is no longer dependent on the presence of the enhancer element.[155] In this case its action was only transient, once established, the active state of the gene is transmitted to daughter cells. On the contrary, when exogenous DNA containing a rearranged IgG gene is transfected into these cells, it still requires an intact enhancer for the formation of the active transcription complex. It is possible that enhancers have in fact a dual function: creation of active initiation complexes and setting up an active chromatin configuration. The in vitro transcription or the transfection experiments detect only one of the facets of the enhancer function.

It is clear that DNA transfer experiments into transgenic animals or flies should help to define the DNA domain that is required for the reproduction of the genuine developmental control of a gene. Experiments with *Drosophila melanogaster* show that P element mediated gene transfer usually 1 kb or more of upstream sequences are required for faithful developmental control of a gene; perhaps more than the sequences required for the control of tissue-specific expression in transient transfection experiments.[93,156] One exception is the hsp 70 gene where 97 bp containing the upstream control element of the promoter are sufficient to confer regulated expression after P element mediated transfer.[157] However, this gene is usually expressed in all tissues and it may in fact lack a developmentally regulated element.

Similar gene transfer experiments undertaken with transgenic mice are not yet conclusive. In most cases where the genuine regulation of the exogenously introduced gene was reproduced at least 0.6 Kb or more of upstream DNA sequences were included in the constructions.[158] Only a recent study of the rat elastase gene shows that 205 bp upstream of the initiation site are already sufficient to assure pancreatic-specific expression.[159] Thus, at least in this case, the promoter upstream sequences that contain

a tissue-specific enhancer can supply the elements that will guarantee tissue-specific expression in the adult animal.[160]

IV. ARE ACTIVE GENES UNDER TORSIONAL STRESS?

The chromosomal DNA of bacteria like *Escherichia coli* is organized into loops or domains. The DNA in these domains is negatively supercoiled as observed both in vitro and in vivo.[161,162] The torsional strain is introduced in the bacterial DNA by an ATP dependent action of the bacterial gyrase (reviewed by Gellert).[163] Toposisomerase I has a reverse action, it relaxes negatively supercoiled DNA. The final degree of supercoiling of the bacterial DNA is obtained by the balance between the action of these two enzymes. Changes in the torsional strain of the bacterial DNA correlate with changes in gene expression.[164]

Eukaryotic chromosomal DNA is also organized in discrete domains or loops attached at their base to some insoluble structure — a matrix.[165-167] A visible example of transcriptionally active loops are the Balbioni rings of chironomus.[142,168] In prokaryotes the DNA in each domain is torsionally stressed as shown by drug intercalation in vitro and in vivo. On the contrary, the same technique does not reveal any significant torsional stress in total chromosomal DNA.[162,169] Only removal of the core histones generate negative superhelical turns in eukaryotic chromosomal or circular viral DNA.[160] However, it cannot be excluded that a small fraction of the chromosome, e.g., highly transcribed regions, are under torsional stress. In fact Luchnik et al.[171] have shown that a small fraction of SV40 minichromosomes may be relaxed by topoisomerase I, perhaps with a concomitant loss of some of its proteins. Furthermore, Harland et al.[172] made the intriguing observation that the circularity of plasmid DNA is required for gene expression in injected xenopus oocytes; cleavage of circular DNA or chromatin by a restriction enzyme or nuclease could relieve torsional stress present in these circular complexes and block transcription. More recent experiments by Ryoji and Worcel[173,174] confirm and extend these observations. They show that circular DNA injected into oocytes is very rapidly assembled into nucleosomes (10 to 30 min) in agreement with previous studies. In a slightly slower time scale (2 hr) they detected a gradual increase in the superhelical density of the template DNA that is correlated with a gradual enhancement in transcription. DNase I and topoisomerase I injections shows that roughly half of the DNA is torsionally strained. This DNA fraction is also relaxed after injection of novobiocin which was shown to exhibit eukaryotic topoisomerase II in vitro. This inhibitor also turns off the transcription of the injected DNA. They hypothesize that the DNA can be assembled into two types of chromatin. A "dynamic" torsionally strained transcriptionally active circular chromosome in which the torsional strain is maintained by continuous action of topoisomerase II. The other half of the DNA is assembled into "static" chromatin, yielding supercoiled DNA only after deproteinization. They suggest that both types of chromatins are associated with core histones, but the nucleosomes of the dynamic chromatin have somehow a modified structure. Consistent with this notion, DNA injected into Xenopus oocytes is assembled into two distinct types of minichromosomes. One is soluble and behaves like conventional chromatin, the other lacks a regular nucleosome repeat and is sensitive to nucleases and generates a half-nucleosome size limit digest with micrococcal nuclease.[174]

The torsional strain observed in active chromatin could generate sites of single stranded DNA (hairpins or B to Z transition zones). It can explain the S1 sensitivity and the reactivity with bromoacetaldehyde of certain DNase I hypersensitive sites.[175,176] Along the same lines Villepontean et al.[177] suggested that removal of the torsional stress by treatment of red blood cells with novobiocin decreases the sensitivity of the globin

gene to DNase I. However, in this case, the authors analyzed the global sensitivity to DNase I after relatively intensive enzyme treatment (10% acid solubility) — a treatment that will introduce many cuts into an active segment of DNA and relieve any topological constraint very early in the digestion. They speculate that the constraint is kept in the active chromatin domain even after multiple nicks or cuts by DNase I due to slow conversion from active to inactive chromatin segments. Perhaps in agreement with the results of Nicolas et al.[178] that showed that partial DNase I sensitivity of active genes are maintained in oligonucleosomes but not in isolated monoparticles.

V. CONCLUSIONS

We can try to summarize a number of features of structural elements that distinguish active from inactive chromatin. We propose that a domain or a loop in the interphase chromosome containing one or several transcribed genes possesses a modified conformation. The ordered nucleosome filament is interupted at several positions by the presence of histone-free DNA segments. Furthermore, it is not folded into a tight solenoid. The switch from inactive to active chromatin structure may involve the binding of one or several control proteins (commitment proteins) to such a domain in its attachment site to the scaffold and inside the loop. Nuclease hypersensitive sites occurring close to the 5′ end of active genes may serve only as entry or action sites for transcription factors (perhaps including topoisomerase) or in addition, they could play a role in the global modification of the chromatin domain. The binding of proteins to these sites could induce modifications of the tertiary structure of the nucleosome filament and of the structure of the nucleosome particle itself. Concomitant with these modifications the chromatin domain could acquire torsional strain. The formation of the active chromatin domain can precede transcription in many cases. The appearance of a second class of regulatory protein, positive transcription factors, or the removal of repressing proteins will switch on the transcription of the gene(s) included in the active domain.

The structural features that distinguish nucleosomes from a committed or actively transcribed domain from inactive domains may in fact be dynamic. The conformational change in the structure of the nucleosome and the higher order structure can be easily lost when nuclei are digested with nucleases especially if torsional strain is involved. Such a dynamic model can explain why it is difficult to pinpoint the difference between nucleosomes containing transcriptionally active or inactive DNA. In addition, the active passage of the RNA polymerase can require further changes in the nucleosome structure such as total melting or even transient removal of the core histones in extremely active genes like rDNA. We favor less a model that requires nucleosome sliding before the coming RNA polymerase — it is difficult to accept it in the case of active convergent transcription that occurs in the histone gene repeat of Drosophila.

It is clear that future research has to focus on characterizing the proteins that bind to certain sites in active chromatin and understanding their function at the domain level. We have to try to identify the mechanisms that control their synthesis and their binding to target sequences only in certain tissues and not in others, and how they switch on processes that result in modifications of the nucleosome particle and of its packaging in higher order structure.

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