

# PLANT CHROMATIN STRUCTURE

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## INTRODUCTION

I am frequently asked the question, "Is plant chromatin just like animal chromatin?" I usually answer, "Yes, no, and we don't know." That is, there are both similarities and differences in chromatin of plants and other eukaryotes. There are some things we know about animal chromatin that are as yet unknown in plant systems, and of course there is much yet to be discovered about chromatin structure in general. The motive for the above-mentioned question is obvious. Much of the knowledge accumulated about chromatin in the past ten years has come from work with animal systems. What we know about plant chromatin is most easily explained in the context of what we know about animal chromatin. Such an approach will be used in this review. In order to keep the review concise, the physical and chemical data obtained from

animal systems will be mentioned only briefly. More extensive comparisons, especially on the basic characterizations of nucleosomes can be found in earlier reviews of plant chromatin structure (69, 83). There are many excellent general reviews (e.g. 6, 10–12, 26–28, 30, 38, 42, 44, 87) that summarize the data which have led to present models of chromatin structure.

A review on chromatin structure might logically begin with a definition of chromatin. In simple terms, chromatin is the material of which chromosomes (both metaphase and interphase) are composed. The exact composition of isolated chromatin depends not only upon the organism and cell type from which it is obtained but also upon the method of isolation. Because molecules that are not true components of chromatin can be trapped or nonspecifically bound during chromatin isolation, it is often difficult to determine whether certain protein or RNA molecules should actually be considered a part of chromatin. In general terms, chromatin consists mainly of (a) about equal parts by weight of DNA and a group of low-molecular-weight basic proteins, the histones, and (b) varying proportions of a diverse group of other proteins which for convenience are called “nonhistone chromatin proteins.”

Before 1974 our ideas of chromatin structure were very unclear, but chromatin was generally viewed as a uniform coil of DNA surrounded by histones and other proteins. In 1974 several lines of evidence crystallized into a subunit concept of chromatin structure with the “nucleosome” as the elementary structural unit (29). Nucleosomes consist of globular cores of eight histone molecules (two each of H2A, H2B, H3, and H4) with 146 base pairs of DNA wrapped in 1.75 turns around the protein core. The fifth histone, H1, interacts with an additional 20 base pairs of DNA to complete two turns of DNA around the histone core. This structure appears to be universal among eukaryotic organisms. The nucleosome cores are then linked by DNA less intimately associated with histones to form structures which when viewed by electron microscopy appear as “beads on a string.” The amount of DNA in the “linker” region varies from organism to organism, cell type to cell type within an organism, and even within chromatin of a single nucleus. This results in an overall nucleosome size (nucleosome repeat) of around 160 base pairs of DNA in some fungi to around 240 base pairs in the sperm of some sea urchins. A “typical” nucleosome as determined for chromatin of most vertebrate animals contains about 200 base pairs of DNA. Nucleosomes presumably contain specific binding sites for nonhistone proteins—the best studied of which are the High Mobility Group (HMG) proteins. An attractive idea is that these proteins bind in a developmentally regulated manner, alter the structure of chromatin, and influence its availability for transcription by RNA polymerases.

The higher order structure of chromatin is not yet clear. However, it is thought that the 100 Å nucleosome fiber undergoes supercoiling to form the circa 300 Å fiber commonly observed by electron microscopy in both inter-

phase and metaphase nuclei. This fiber can then undergo further coiling and condensation and, perhaps with the involvement of a protein scaffold, result in the structures observable by light microscopy as mitotic chromosomes (26).

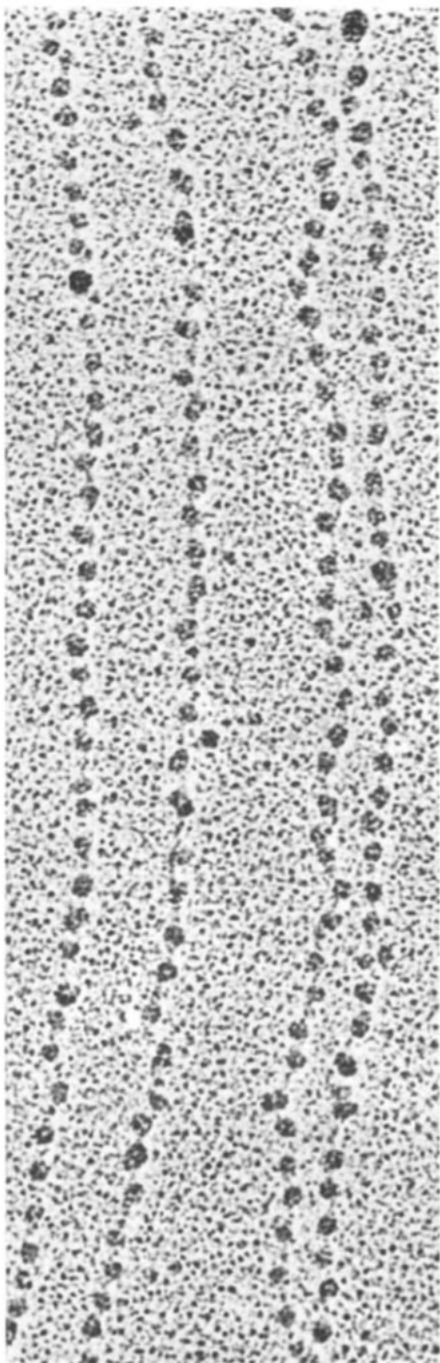
## NUCLEOSOMES IN HIGHER PLANTS

### *Microscopic Investigations*

The first electron micrographs that showed a regular repeating subunit structure of chromatin were published in 1974 by Olins & Olins (54), who used material from rat thymus and liver and from chicken erythrocytes. The first similar electron micrographs showing nucleosome-like material in plants were presented by Nicolaieff et al (53) and by Gigot et al (20) in 1976. The nucleosomes from tobacco leaf nuclei were estimated by these workers to have a diameter of about 160 Å, which was somewhat larger than the 125 Å reported by Oudet et al (56), who used a similar technique for nucleosomes of various vertebrate animals. The size differences were not considered significant and were attributed to variations in granularity of platinum shadowing. Since that time, several electron micrographs of plant nucleosomes have appeared (e.g. 39, 48), including those from roots of germinating corn embryos presented by Greimers & Deltour (21; Figure 1). The diameter of the corn nucleosomes was estimated to be 125 Å, the same as those of vertebrate animals. At the level of the electron microscope, there is no reason to believe that plant nucleosomes have a structure that is different from that of nucleosomes of vertebrate animals.

### *Biochemical and Biophysical Investigations*

A wide variety of biochemical and biophysical approaches have provided evidence for a nucleosome structure for chromatin. These approaches cannot all be detailed here (see reviews mentioned in the Introduction), but the principal evidence has been: (a) the generation of "nucleosome ladders" upon digestion of chromatin with micrococcal nuclease; (b) a limit micrococcal nuclease digestion product with a sedimentation coefficient of about 11S containing about 146 base pairs of DNA and two each of the four "nucleosomal histones" H2A, H2B, H3, and H4; (c) generation of a series of single-stranded DNA fragments differing in length by multiples of about 10 bases upon digestion of chromatin with DNase I, indicating that DNA lies on the outside of the nucleosome; and (d) the demonstration that the nucleosomal histones interact specifically with one another to form a globular, multisubunit core for the nucleosome and that this core and DNA can be used to reconstitute nucleosomes in vitro. Higher plants have been shown to have canonical nucleosomes by all these criteria. An example of a micrococcal nuclease ladder generated from plant chromatin is shown in Figure 2.



*Figure 1* Electron micrograph of nucleosomes from roots of germinating corn embryos show typical "beads on a string" appearance. The mean diameter of nucleosomes is  $125 \pm 13$  nm. (Data of Greimers & Deltour (2))

*Figure 2* Micrococcal nuclease "ladder" of wheat embryo chromatin. Nuclei isolated from wheat embryos were adjusted to 1 mg/ml DNA/ml and digested for 1 minute at 37°C with 50 units of micrococcal nuclease/ml. DNA fragments were separated on non-denaturing agarose gels and visualized under UV after staining with ethidium bromide as previously described (76). The nucleosome repeat size is 186 base pairs.

In 1975 McGhee & Engel (43) presented the first nucleosome ladder generated with plant material. These workers estimated a repeat size of 170  $\pm$  30 base pairs of DNA for pea chromatin. Since this report, the nucleosome repeat has been estimated for a number of different species by a number of different workers (7, 19, 22, 33, 34, 36, 45, 46, 49, 50, 59) and has always been reported in the range of 170 to 200 base pairs. In most cases the repeat values have not been shown to differ in different plant tissues or in different stages of development (22, 33, 36, 50); however, Murray & Kennard (50) have documented a striking contrast in the repeat size in bean leaves (191 base pairs) and in a tissue undergoing endopolyploidy, developing cotyledons (177 base pairs). These changes are in the same range as those found by Weintraub (85) in the course of erythropoiesis in the chick. The changes in repeat in chick are accompanied by dramatic changes in the content of the H1-like histone, H5, in chromatin. Whether such changes occur in beans is as yet undetermined. However, changes in relative quantities of H1 histones during pea cotyledon development were reported long ago (17).

A number of workers have isolated plant nucleosomes on sucrose gradients and investigated their physical and chemical properties. Gigot et al (20) separated micrococcal nuclease-generated mono-, di-, and trinucleosomes from tobacco and measured the sedimentation coefficient of the monomer to be 12.9S. The monomer contained all the non-H1 histones in a 1:1 ratio with DNA. Frado et al (19) did similar work with pea chromatin and determined a monomer sedimentation coefficient of 11S. Yakura et al (92) estimated monomer sedimentation coefficients of 11S for *Vicia faba* and *Trillium kamtschaticum*. Mithieux & Roux (45) determined a value of 10.6S for barley mononucleosomes. Philipps & Gigot (59) demonstrated the existence of a circa 140 base pair fragment of DNA after extensive digestion of tobacco and barley chromatin with micrococcal nuclease. Grellet et al (22) obtained a "core" DNA size of 145 base pairs for pea nucleosomes. A 10-base "ladder" of single-stranded DNA fragments resulting from DNase I digestion of plant chromatin has also been well documented (7, 22, 34, 59).

Physical studies undertaken with plant chromatin include the circular dichroism and electric birefringence work of Mithieux & Roux (45). In their previous work with rat liver chromatin (40), a transition from negative birefringence for monomer through pentamer to positive birefringence for heptamers and above was correlated with a higher order structure for chromatin involving a repeating structure of six nucleosomes (90). Preliminary observations were similar for their work with barley chromatin (45). Monomer through trimer nucleosomes isolated from sucrose gradients displayed negative steady-state birefringence values. Higher oligomers isolated from the bottom of the gradient showed positive values, and oligomers isolated from the middle of the gradient (including hexamers) had a birefringence value of zero.

Transient electric birefringence decay curves were used to measure relaxation times for the various oligomers and compare them to their rat liver counterparts. Values for trimers and high oligomers were essentially the same in barley and rat liver. However, the relaxation times were significantly longer in barley dimers than in rat dimers, indicating a more elongated structure. The physical basis for this difference is unexplained, although it does correlate with a lower sedimentation coefficient for barley dimer nucleosomes as compared with those of the rat (45). Plant H2A and H2B histones appear to be significantly larger than those of animals (see section on Histones); however, it is unclear how this could result in dimer nucleosomes of significantly more elongated form. The circular dichroism spectrum of unfractionated barley chromatin was typical of that of chromatin of vertebrate animals (45) in having positive peaks at 272 nm and 282 nm and a negative peak at 295 nm. Monomer nucleosomes isolated from vertebrate animals have a similar circular dichroism spectrum (35). Barley mononucleosomes have lost the 272 nm peak and the 295 nm peak, and the molar ellipticity at 282 nm has increased by over 50%. Such a spectrum probably denotes a less constrained structure for barley than for animal mononucleosomes (45). Isolated yeast mononucleosomes have been demonstrated to have a circular dichroism spectrum similar to that of barley (35).

Although plant histones H3 and H4 are essentially identical to their animal counterparts, plant H2 histones have distinct chemical and physical properties (see section on Histones). Despite the differences in H2 histones, plant H2A, H2B, H3, and H4 interact with each other to form nucleosomes in exactly the same way as do animal histones (41, 71). Furthermore, plant and animal histones are interchangeable in the pairwise interactions that stabilize the nucleosome (72), indicating evolutionary conservation of histone-histone binding sites. Additional evidence for the importance of the evolutionary stability of the histone-histone contacts in nucleosome formation comes from reconstitution studies. Wilhelm et al (89) and Liberati-Langenbuch et al (37) reconstituted nucleosome cores using combinations of histones from plants (corn or tobacco) and animals (chicken). The success of these reconstitutions demonstrates that the essential histone-histone contacts have been evolutionarily conserved even though there are distinct differences in plant and animal H2A and H2B histones.

## CHROMATIN PROTEINS

### *Histones*

Histones have a confusing and hoary history. A plethora of nomenclatures have been used to describe them, and we have gone from a period of thinking of an almost unlimited multiplicity of histones through an assuring period when there were "only five" histones to our present thinking that there are but five major

classes of histones but variants within each class (27). The early work of Fambrough & Bonner (14–17) laid the foundations for characterizing plant histones. They demonstrated the limited heterogeneity of plant histones, pointed out their similarity to animal histones, and showed that plant histones, like animal histones, could be divided into three groups according to content of basic amino acids: (a) the very lysine rich, now known as H1; (b) the slightly lysine rich, now called H2A and H2B; and (c) the arginine rich, now called H3 and H4.

As reviewed by Isenberg (27), the work of Fambrough & Bonner led to the finding of extreme evolutionary conservation of the arginine-rich histones. Both pea and cow H4 have 102 amino acid residues. Their sequences are identical at 100 positions, and the two substitutions are conservative ones. Histone H3 is nearly as conserved, having sequence identity in 131 of 135 positions. Histones H2A and H2B are less conserved. Differences in amino acid composition, electrophoretic mobility (see Figure 3), and behavior in histone fractionation schemes (1, 4, 5, 18, 25, 31, 32, 45, 46, 51, 52, 55, 58, 63–67, 73, 74, 77, 80) have led to uncertainty about whether the slightly lysine-rich plant histones should properly be referred to as “H2A” and “H2B.” The reason for the uncertainty is that the histone classes are defined on an *operational* basis (how they are isolated and purified) not on the basis of physiological *function*. There are no easily assayable biological functions by which the histone classes can be identified.

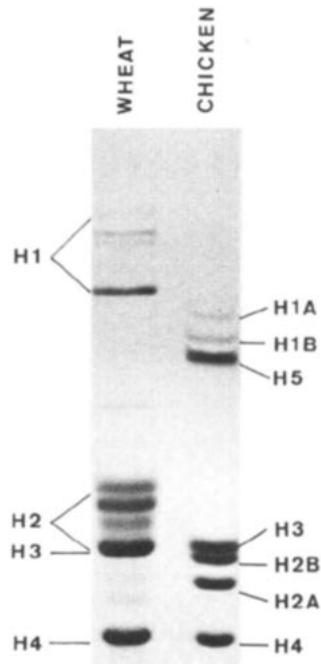


Figure 3 Electrophoretic comparison of histones from wheat embryos to those of chicken erythrocytes on sodium dodecylsulfate polyacrylamide gels. The plant H3 and H4 histones comigrate with their animal counterparts. The H2 histones are less evolutionarily conserved. The plant proteins have greater apparent molecular weights than their animal counterparts, and both H2A and H2B appear as multiple bands of distinct electrophoretic mobility as compared to single bands for H2A or H2B from the animal system. Histones were isolated and analyzed as previously described (68).

Although the complete biological function of the histones is not known in detail, the observation that the H2A, H2B, H3, and H4 histones form the globular protein core of the nucleosome and the observation of a pattern of specific pairwise interactions between calf thymus histones (8) suggest a *quasi-functional* definition of the histone classes. D'Anna & Isenberg (8), using the techniques of fluorescence anisotropy, light scattering, and circular dichroism, showed that in solution strong interactions occur between the histone pairs H3-H4, H2B-H4, and H2A-H2B. Thus, H2B can be defined as the slightly lysine-rich histone that interacts strongly with H4. Conversely, H2A can be defined as the slightly lysine-rich histone that does not interact strongly with H4. Experiments using these techniques with pea histones showed clearly that the same pairwise interactions occur with plant histones (71) that occur with animal histones. Thus, despite the differences in plant and animal slightly lysine-rich histones, the designations "H2A" and "H2B" are justified for the plant histones.

Not only is the pattern of pairwise interactions evolutionarily conserved, but the association constants of the interactions are the same for the plant and animal histone pairs. Furthermore, interkingdom hybrid histone complexes can be formed between calf and pea histones (72), which also display the same association constants as those observed when the histones of calf or pea alone are used. Thermodynamic arguments led to the prediction that the surfaces involved in the histone-histone interactions must have highly (if not completely) conserved amino acid sequences (72). A further prediction of a large interaction surface was made based on the observation that upon complexing there was a large increase in the number of amino acid residues assuming an  $\alpha$ -helical conformation. Some of these predictions were confirmed by Martinson & True (41), who showed by cross-linking experiments that the same histone-histone contacts demonstrated for histone pairs in solution actually occur *in vivo*, and the amino acids at the sites of contact are evolutionarily conserved.

Despite the great effort that has gone into sequencing histone molecules (27), to date no plant histone molecule has been completely sequenced except for H3 and H4. Some sequencing has been done, however (23, 61, 84), and the general conclusion has been that the middle and carboxy terminal portions of the H2 histones (the portions involved in histone-histone interactions) are highly conserved. For example, the carboxy terminal of a mixture of wheat H2B histones has been sequenced (84) with the finding that of 89 residues sequenced, 69 positions are identical to those of calf thymus H2B histones. Most of the substitutions are conservative ones.

In addition to the differences in electrophoretic mobility, amino acid compositions, and fractionation properties of plant and animal H2 histones mentioned above, plant H2 histones differ from their animal counterparts by an

additional salient feature—the characteristics of the variants or subtypes within a histone class. Animal histone variants differ mainly by amino acid point substitutions (27), although minor variants of different molecular weight do occur (88). In plants the major histone variants are molecular weight variants (31, 68). For example, wheat has six forms of histone H2B which elute differentially in exclusion chromatography, and according to their distinct mobilities on sodium dodecylsulfate polyacrylamide gels, have estimated molecular weights of from 15,300 to 19,000 daltons (68). Wheat H2A has three major variants ranging from 16,600 to 19,000 daltons in molecular weight. The three H2A variants are present in about equimolar proportions. In addition to amino acid analysis and peptide mapping (68), partial sequence analysis (61) indicates that these histone variants are not degradation artifacts.

Do the histone variants have special physiological functions, or do they simply represent allowable variations in the structure of proteins of multigene families? There are many clues that indicate their possible involvement in the regulation of gene expression (see 69 for discussion), and certainly the extent of histone variability provides the potential for extensive nucleosome heterogeneity; however, we have as yet no solid evidence that different types of nucleosomes, based on their histone variant content, have any special role in chromatin structure.

The most variable of the histones are the H1 histones (27, 67, 77). Both plant and animal H1 variants exist that differ not only in amino acid sequence but also in molecular weight. Very little sequence work has been carried out with plant H1 histones. The only reported work is that of Hurley & Stout (25), who sequenced about 25% of one of the H1 variants of maize. Maize H1 histones, like other plant H1 histones, appear to have higher molecular weights than their animal counterparts. Despite this difference, the partial characterization by Hurley & Stout demonstrated that plant H1 is organized in generally the same way as animal H1. That is, it appears to have three structural domains: (a) a random coil, basic N-terminal region, (b) a central globular region, and (c) a highly basic C-terminal region. The central globular section seems to have a high degree of sequence conservation in animal H1 (3). This maize region has not been completely sequenced, but the residues that have been established are highly conserved. Cross-linking data, which show this region in contact with specific regions of H2A in animals, have led Boulikas et al (3) to conclude that the H1-H2A contacts are evolutionarily conserved and serve to close the ends of DNA around the nucleosome.

### *Histone Genes*

In a 1981 review on the organization of histone genes, Hentschel & Birnstiel (24) pointed out that the histone genes of plants “have not been investigated and thus they represent a clear gap in our knowledge of histone gene structures.”

Since that time some progress with plant histone genes has been made. Padayatty and coworkers have used cell-free translation to identify histone mRNAs from the 9-12S poly A<sup>-</sup> RNA fraction of rice embryos (2). They have used this RNA to identify a cloned DNA fragment that contains the genes for one form of H2A, H2B, and H4 and have established that the transcription of these genes is bidirectional (81, 82). Sequences of these genes, the organization of other histone genes in rice, and the extent of histone gene repetition in rice have not yet been reported by these workers. Tabata et al (79) have taken a different approach in order to characterize a histone H4 gene from wheat. They have isolated a segment of DNA containing the wheat H4 gene from a Charon 4 wheat genomic library using cloned sea urchin histone H4 DNA as a probe. The sequence of the H4 gene shows that it contains no intervening sequences and codes for a protein identical in sequence to H4 from peas. Additionally they have estimated a repetition frequency of 100–125 copies of the H4 gene per hexaploid wheat genome.

### *High Mobility Group Proteins*

Nonhistone chromosomal proteins have long been suspected of being involved in the control of gene expression in plants as well as in animals (83). Of animal nonhistone chromosomal proteins, the High Mobility Group (HMG) proteins are by far the best studied (28, 87). The current excitement concerning these proteins is based on considerable evidence that they are structural proteins of nucleosomes in transcriptionally active chromatin. In brief, the evidence for this is: (a) HMG proteins are released from nuclei treated with DNase I under conditions in which actively transcribed genes are preferentially digested by this endonuclease; (b) chromatin from which HMG proteins have been removed loses the preferential sensitivity of active genes to DNase I; (c) the preferential sensitivity can be restored by reconstituting the chromatin with purified HMG proteins; (d) HMG affinity columns have been used to isolate mononucleosomes highly enriched in transcribed sequences; and (e) in chromatin fractionation schemes based on light digestion with micrococcal nuclease, HMG proteins coisolate with the "active" fraction (see 87 for review).

As with the histone classes, HMG proteins are *operationally* defined. First noticed as contaminants of H1 histone preparations from calf thymus (28), they were defined according to the criteria used to isolate and purify them. That is: (a) HMG proteins can be extracted from purified chromatin with 0.35 M NaCl (a concentration lower than that which will extract histones), and (b) they are soluble in 2% trichloroacetic acid. These proteins have higher electrophoretic mobilities than the chromosomal proteins extracted with 0.35 M NaCl but which are *insoluble* in 2% trichloroacetic acid; thus, their designation as "high mobility group proteins." The four calf thymus HMG proteins have very unusual amino acid compositions. They consist of about 50% charged res-

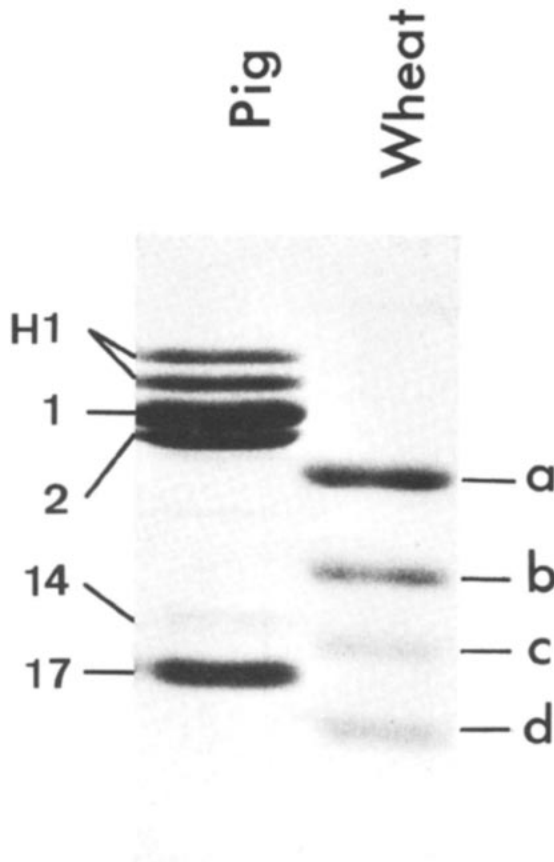
idues (25% acidic and 25% basic) and are relatively high in proline (7% or more). The HMG proteins are not as evolutionarily conserved as the histones.

As explained above, we have some general ideas on the roles of these proteins, but we know of no easily assayed biological function. Thus, it is difficult to determine if organisms and tissues other than calf thymus have proteins that should be termed HMG proteins. For example, another vertebrate animal, trout, has a protein that is almost certainly an HMG according to many criteria, including striking sequence homology with calf thymus HMG proteins 14 and 17. Yet this protein has only about half the characteristic content of acidic amino acids (9). Without the sequence information, this protein's identification as an HMG would be questionable.

The problem is of course more severe in distantly related organisms (such as plants) which may have proteins that fulfill the biological roles of HMG proteins but are much lower in sequence homology. Wheat embryos have four proteins that meet the operational criteria of being HMG proteins (70, 75). The wheat proteins have the same general electrophoretic mobilities as HMGs from mammalian tissues, but no plant fraction exactly comigrates with any animal fraction (Figure 4). Three of the proteins HMGb, c, and d have the content of basic and acidic amino acid residues characteristic of calf thymus HMG proteins, but none of these has as much as 7% proline. The wheat embryo protein that we have called HMGa is high in proline and basic amino acid residues. However, like the trout HMG protein, it has only about half the characteristic content of acidic amino acid residues. Peptide maps of the wheat HMG proteins have little in common with those of HMGs of vertebrate animals (70). In support of the identification of the wheat proteins as HMG proteins are the observations that they are released from nuclei treated with DNase I (76) and cofractionate with putatively active chromatin fractions generated with micrococcal nuclease (70). Four proteins have been isolated from barley which are extracted from chromatin with 0.35 M NaCl and are soluble in 2% trichloroacetic acid (46). These proteins are of higher molecular weight than the wheat HMG proteins but have not been further characterized. They do cofractionate with the earliest nucleosomes solubilized from chromatin by micrococcal nuclease treatment. Proteins that meet the operational criteria for being HMG proteins have also been isolated from maize seedlings (57).

## STRUCTURE OF ACTIVE CHROMATIN AND SPECIFIC GENES

In animal systems, actively transcribed genes have a chromatin structure different from that of bulk chromatin (10-12, 42, 69, 87). The approach most often used to study the altered conformation depends on the increased sensitiv-



*Figure 4* Electrophoretic comparison of High Mobility Group (HMG) proteins from wheat embryos to those of pig thymus on sodium dodecylsulfate polyacrylamide gels. The wheat proteins have similar electrophoretic mobilities to those of pig thymus, but no wheat HMG comigrates with any pig thymus HMG. The apparent molecular weights of the wheat proteins based on mobilities relative to common standards are: HMGa = 24,300; HMGb = 20,400; HMGc = 17,400; HMGd = 14,500 (70). HMG proteins were isolated and analyzed as previously described (70).

ity of active or potentially active genes to various nucleases, predominantly DNase I. Three levels of enhanced DNase I sensitivity of active chromatin over bulk chromatin have been recognized: 1. Large domains of chromatin that contain transcribed genes and several kilobases of flanking sequences are more readily digested by DNase I than is the bulk of chromatin. This has generally been attributed to the presence of an "open" chromatin structure perhaps mediated by the loss of H1 histones. 2. Yet more sensitive to DNase I are the actual transcribed genes and their more immediate flanking sequences. There is

evidence that this enhanced nuclease susceptibility results from the presence of HMG proteins or other specific nonhistone chromatin proteins (60). 3. Relatively specific sites termed "DNase I hypersensitive sites" often occur within 1000 base pairs of the 5' ends of genes that are active or can be activated. The presence of these hypersensitive sites has often been attributed to a local absence of nucleosomes perhaps accounted for by the binding of specific proteins (13, 91). DNase II and micrococcal nuclease preferentially solubilize chromatin that is actively being transcribed (87), and the single strand-specific nuclease S1 has been shown to attack sites in active chromatin and in supercoiled plasmid DNA which correspond closely to DNase I hypersensitive sites (86).

Several studies on nuclease sensitivity of plant chromatin have been carried out (34, 36, 46, 47, 50, 76, 78). These studies have in general supported the idea that active chromatin in plants is more susceptible to nuclease digestion than is bulk chromatin. However, in only a few studies have the conclusions been supported by data derived from the use of hybridization probes representing products of transcription. In one study (76), solution hybridization was used to demonstrate that in DNase I-treated wheat embryo nuclei, the sequences homologous to polysomal poly A<sup>+</sup> RNA were more rapidly degraded than was bulk DNA. In the course of the digestion, there was preferential release of the four wheat proteins which have been referred to above as HMG proteins.

Murray & Kennard (50) have investigated the chromatin structure of the phaseolin gene family in French bean. Expression of these genes is under strict developmental control. Messenger RNA and protein are first detectable in midmaturation stage and continue to accumulate through the maturation of cotyledons. Neither the protein nor mRNA is detectable in leaves. Using Southern blotting procedures and probes to the DNA within the coding sequences of the gene, and to DNA up to 1400 base pair upstream from the 5' end of the gene or 1000 base pairs downstream from the 3' end of the gene, these workers were able to demonstrate that phaseolin genes are more sensitive to DNase I digestion than is bulk chromatin, and the genes are more sensitive in tissues where they are expressed (cotyledons) than where they are not expressed (leaves). It was mildly surprising that no evidence for DNase I *hypersensitive* sites could be found. In correlation with the lack of observed DNase I hypersensitive sites in the phaseolin genes, Murray & Kennard found no evidence for S1 nuclease hypersensitive sites in chromatin. They did, however, find specific S1 sites in four cloned genes when they were in supercoiled plasmids. It is far too early to generalize, but it will be interesting to determine whether DNase I hypersensitivity is in fact a common feature of actively transcribed plant genes.

Ribosomal RNA genes, which are present in high copy number, have been

shown not only to lack enhanced sensitivity to DNase I, but to actually be less susceptible to digestion than the bulk of DNA (34, 36).

The chromatin structure of foreign genes incorporated into higher plant genomes is of interest not only from a purely biological standpoint but also because of its relevance to genetic engineering. Does foreign DNA assume a nucleosome structure when it is incorporated into the host genome? If so, does it take on the specific chromatin structure (histone variant composition, HMG content, 5-methylcytosine content, nuclease sensitivity, higher order structure, etc) existing at the site of incorporation? Does a specific chromatin structure influence the expression of foreign DNA? Does chromatin structure at a particular site in the host determine if foreign DNA can be incorporated at that site? Most of these questions have not yet been approached.

However, in at least one case evidence has been presented that foreign DNA does take on a nucleosome structure upon incorporation. Schäfer et al (62) have used micrococcal nuclease to treat nuclei isolated from tobacco suspension cultures transformed with *Agrobacterium tumefaciens*. DNA of nucleosomes isolated from sucrose gradients was separated on agarose gels, transferred to nitrocellulose, and probed with nick-translated restriction fragments representing the entire T-DNA region. The probes hybridized to the nucleosomal DNA and the pattern on autoradiograms appeared to be identical to the nucleosome ladders of total chromatin obtained by ethidium bromide staining. The probes did not react with nucleosomal DNA isolated from habituated but nontransformed tobacco suspension cultures. No control of micrococcal nuclease-digested, deproteinized total DNA was presented. However, restriction fragments of cloned DNA representing the T-DNA did not give a nucleosome pattern. The data presented support the conclusion that at least some of the incorporated T-DNA is organized into nucleosomes, but it is difficult to tell what proportion.

The authors probed only DNA in the monomer through trimer nucleosome sizes. Thus, a considerable amount of DNA was not tested. Also, the validity of the contention that the nucleosome repeat size of the T-DNA is the same as that of bulk DNA is difficult to evaluate based on a nucleosome ladder that goes only up to tri-nucleosomes. S. Gelvin (personal communication) has carried out similar experiments but has used *total* DNA resulting from micrococcal nuclease digestion to hybridize to the T-DNA probes. Using total DNA, Gelvin is unable to find evidence for a nucleosomal organization for T-DNA. Furthermore, he finds no evidence for a nucleosomal organization of DNA complementary to ribosomal RNA (rDNA) in the transformed tissues, despite the fact that rDNA can be clearly shown to be organized into nucleosomes in nontransformed tissues. Ethidium bromide staining also clearly shows that the bulk of the DNA is organized into nucleosomes in both transformed and nontransformed tissues. These specific issues need to be resolved by further

work, and the whole area of chromatin structure of foreign DNA incorporated into host genomes appears to have an interesting future.

## SUMMARY

Dramatic progress has been made over the last ten years toward an understanding of chromatin structure in eukaryotic organisms. Most of the work has been carried out with animal systems (especially vertebrate animals), and what we have discovered about the chromatin of higher plants is viewed against that background. The fundamental chromatin structure in plants is the same as that in animals, even though some differences have already been pointed out and clues have been found that may lead to the discovery of other and perhaps quite significant differences.

The existence of a nucleosome structure in plants has been demonstrated by electron microscopy and by a number of biochemical and biophysical techniques. The nucleosome repeat size in plant chromatin is in the same range as that considered typical for vertebrate animals. The DNA content of the nucleosome core is the same in plants and animals. The nucleosome core contains two each of the four nucleosomal histones H2A, H2B, H3, and H4, and is stabilized by the same histone-histone interactions that occur in animal systems. Plant H3 and H4 histones are highly evolutionarily conserved and are essentially the same molecules in plants and animals. The H2 histones are less conserved. Plant H2A and H2B are larger than their animal counterparts. They have distinct amino acid compositions and are made up of families of closely related variants that differ in molecular weight.

The chromatin structure of potentially transcribable genes in plants renders the DNA more susceptible to digestion by DNase I than is DNA in bulk chromatin. However, in the one gene investigated thus far, phaseolin, no evidence of DNase I *hypersensitive* sites has been found. Plant chromatin contains proteins which meet operational criteria to be termed HMG chromatin proteins. These proteins are released from nuclei treated with DNase I and fractionate with "active" chromatin obtained by micrococcal nuclease digestion. Plant and animal HMG proteins share some chemical and physical properties but they differ to a much greater extent than do plant and animal histones. Whether the plant HMG proteins have the same biological roles as the animal HMG proteins has yet to be determined.

The scope of this review has been limited intentionally. Several aspects of higher plant chromatin have not been discussed, such as higher order structure of plant chromatin and chromosomes, nonhistone chromatin proteins other than the HMGs, modification of chromosomal proteins such as acetylation, phosphorylation, and poly(ADP-ribosylation), methylation of plant DNA, Z-form DNA in plants, synthesis of DNA, histones and histone variants during the cell

cycle, changes in the template activity of chromatin during development, and changes in various chromatin-associated enzyme activities during development. Studies of these topics as well as many of those discussed in the review are in their infancy, but the groundwork is being laid for future studies which potentially will result in a more sophisticated understanding of the basics of plant chromatin structure and eventually lead to an understanding of its involvement in the developmental regulation of gene expression in higher plants.

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