The Arabidopsis Adh Gene Exhibits Diverse Nucleosome Arrangements within a Small DNase I-Sensitive Domain

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The alcohol dehydrogenase (Adh) gene from Arabidopsis shows enhanced sensitivity to DNase I in cells that express the gene. This generalized sensitivity to DNase I is demarcated by position -500 on the 5' side and the end of the mRNA on the 3' side. Thus, the gene defined as the promoter and mRNA coding region corresponds very closely in size with the gene defined as a nuclease-sensitive domain. This is a remarkably close correspondence between a sensitive domain and a eukaryotic transcriptional unit, because previously reported DNase I-sensitive domains include large regions of DNA that are not transcribed. Nucleosomes are present in the coding region of the Adh gene when it is expressed, indicating that the transcriptional elongation process causes nucleosome disruption rather than release of nucleosomes from the coding region. In addition, the regulatory region contains a loosely positioned nucleosome that is separated from adjacent nucleosomes by internucleosomal DNA segments longer than the average linker DNA in bulk chromatin. This specific array of nucleosomes coexists with bound transcription factors that could contribute to the organization of the nucleosome arrangement. These results enhance our understanding of the complex interactions among DNA, nucleosomes, and transcription factors during gene expression in plants.

INTRODUCTION

Eukaryotic genes are expressed in different tissues with precisely defined patterns during development and in response to extracellular stimuli. This refined regulation involves a variety of mechanisms, including the interaction of transcription factors with DNA regulatory sequences (Buratowski, 1994; Tjian and Maniatis, 1994), and is strongly influenced by the complex structure of DNA in the nucleus (Felsenfeld, 1992).

The eukaryotic genome has several levels of chromatin organization. The simplest level is represented by the nucleosome, which is composed of 160 to 200 bp of DNA associated with a core of histones (Clark and Kimura, 1990). An array of nucleosomes is organized into a helical structure with a diameter of 30 nm, referred to as the chromatin fiber (Gasser and Laemmli, 1987). Specific DNA sequences of this fiber, called scaffold attachment regions (SARs), bind to the nuclear scaffold to generate loops up to hundreds of kilobases long that distribute chromatin along the chromosome (Mirkovitch et al., 1984).

Many reports show that eukaryotic genes are generally more sensitive to DNase I when they are expressed. Their sensitivity is believed to reflect an open conformational state of chromatin. This generalized sensitivity to DNase I extends kilobases proximally and distally to the coding regions (Weintraub and Groudine, 1976; Bellard et al., 1980; Stalder et al., 1980; Storb et al., 1981; Lawson et al., 1982; Wood and Felsenfeld, 1982; Murray and Kennard, 1984). Although several specific characteristics of transcriptionally active chromatin have been described (van Holde et al., 1992), the cause for the increased sensitivity to DNase I as well as the significance of the extended sensitive domains have yet to be explained fully.

Nucleosomes have been reported to be absent (Conconi et al., 1989) and present (van Holde et al., 1992) in the coding region of transcriptionally active genes. When they are present, certain biochemical modifications of the histones have been described (van Holde et al., 1992). However, the biological significance of these modifications is unclear. To explain the presence of nucleosomes in genes that are transcribed, several models involving progressive displacement of nucleosomes by the elongating RNA polymerase have been proposed (van Holde et al., 1992; Adams and Workman, 1993).

DNase I-hypersensitive sites in the 5' flanking region of transcriptionally active genes are associated with the presence of transcriptional regulatory factors and with the release of nucleosomes (Elgin, 1988). Nucleosomes may play an essential role in the regulation of transcriptional initiation. In some cases, nucleosomes sterically inhibit the interaction of transactivating proteins with their target sequences (Piña et al., 1990) and need to be released or disrupted to allow transcriptional activation (Almer et al., 1986; Richard-Foy and Hager, 1987). In other cases, nucleosomes remain bound to transcriptionally active promoters and facilitate the interaction of regulatory factors that bind to distant target sequences (Thomas and Elgin, 1988).
1988). In all of these cases, nucleosomes seem to regulate gene expression by affecting the accessibility, distribution, or spatial arrangement of transcriptional regulatory factors and thereby influence the formation of hypersensitive sites.

Evidence that nucleosome organization changes with gene activation in plants is limited (Murray and Kennard, 1984; UlI and Franco, 1986). However, there is extensive documentation of changes in chromatin structure correlated with gene activation in plants (Spiker et al., 1983; Ashraf et al., 1987; Kaufman et al., 1987; Paul et al., 1987; Wurtzel et al., 1987; Frommer and Starlinger, 1988; Götz et al., 1988; Thompson and Flavell, 1988; Lund et al., 1995).

We have analyzed the chromatin structure of the alcohol dehydrogenase (Adh) gene from Arabidopsis. This study was designed to approach general issues of chromatin structure and gene expression in Arabidopsis. The Adh gene is well suited for this purpose because many aspects of its transcriptional regulation are known already. This single-copy gene is induced by hypoxia, low temperatures, and dehydration in the roots of Arabidopsis and is expressed constitutively in cell cultures (Dolferus et al., 1985, 1994; Chang and Meyerowitz, 1986; Ferl and Laughner, 1989). In contrast, Adh is not expressed in mature aerobic leaves (McKendree et al., 1990). Several sequences 5' of the start of transcription that interact with nuclear factors in cultured cells have been identified by in vivo footprinting (Ferl and Laughner, 1989). These sequences include parts of the anaerobic response element (Ferl and Nick, 1987; Walker et al., 1987) and several G-boxes and G-box-like sequences found in many eukaryotic promoters (Williams et al., 1992). Three G-box binding factors and a family of proteins associated with the G-box binding complex (GF14) have been described in Arabidopsis (Lu et al., 1992; Schindler et al., 1992). In addition, deletion analyses of the 5' region have revealed functional elements that are important for the expression of the Adh gene (McKendree and Ferl, 1992; Dolferus et al., 1994).

We show in this report that the Adh gene constitutes a small DNase I-sensitive domain that is defined by the boundaries of the Adh coding and regulatory regions. When the gene is active, nucleosomes in the regulatory region of the gene are in a specific altered configuration, unlike a normal nucleosome array in Arabidopsis. In addition, the elongating RNA polymerase displaces, rather than releases, the nucleosomes within the coding region of the gene.

RESULTS

Sensitivity of the Adh Gene to DNase I

The sensitivity of the Adh gene to DNase I was studied in four DNA regions within and around the gene. The positions of the regions as well as the location of probes used in this study are presented in Figure 1. The 5' upstream distal (5'UD) region extends from -2000 to -847. The 5' upstream (5'U) region extends from -847 to +412 and contains the promoter of the gene. The 3' downstream (3'D) region extends from +1130 to +2236. This region contains a significant portion of the transcribed sequences and the 3' end of the mRNA, located at position +1965. The 3' downstream distal (3'DD) region extends from +2236 to +2800.

Nuclei from Arabidopsis cultured cells, where the Adh gene is highly expressed, and from mature leaves, where Adh is not expressed (McKendree et al., 1990), were digested with increasing concentrations of DNase I. The resulting DNA was purified and digested to completion with EcoRI, resolved in agarose gels, and stained with ethidium bromide (Figure 2, bottom). The DNA was transferred to nylon membranes. These membranes were hybridized to the probe designed to study the 5'U region using the indirect end-labeling technique (Figure 1; Wu, 1980). The resulting autoradiograms are shown at the top of Figure 2. At left and right are leaves and cultured cell samples, respectively. The marker lane in this figure contains genomic DNA digested with several restriction enzymes; it was used to map the position of the DNase I-sensitive areas. Two lanes, one from cultured cells and the other from leaves, were selected to quantitatively compare the sensitivity to DNase I of their 5'U regions. These samples were selected on the basis of equal DNase I activity (as determined by digestion of bulk genomic DNA, as shown in Figure 2, bottom) rather than on units of DNase I used. As often happens, nuclei from different plant tissues required different amounts of DNase I to receive equivalent digestion. The samples selected for study are indicated with triangles at the bottom of Figure 2. (The DNAs that appear in the indicated lanes were also used to generate subsequent analyses of the regions shown in Figure 3.) Comparison of equally digested nuclei from leaves and cultured cells clearly shows that the DNA between approximately positions +1 and -495 is hypersensitive to DNase I in cultured cells.

The sensitivity to DNase I of the 5'UD, 3'D, and 3'DD regions of the Adh gene was also studied (Figure 3). The blots in Figure 2 were stripped and rehybridized to the probes designed...
to analyze the 3'D and the 3'DD regions (see Figure 1). The lanes selected to compare the sensitivity of these regions are shown in Figure 3 and correspond with the lanes marked by triangles in Figure 2. To study the 5'UD region, the same DNase I-digested samples that formed the lanes marked by triangles in Figure 2 were digested with EcoRV and hybridized with probe I (see Figure 1). Thus, the same samples used to analyze the sensitivity of the 5'U region were used to analyze the sensitivity of the 5'UD, 3'D, and 3'DD regions. The left lanes of each gel in Figure 3 show leaf samples, the center lanes show cultured cell samples, and the right lanes show the markers used for mapping. Figure 3 shows that the sensitivity to DNase I of the 3'D region is higher in cultured cells than in leaves. On the other hand, the sensitivity of the 5'UD and the 3'DD regions is similar in leaves and in cultured cells.

Densitometric scans of the lanes selected to analyze the sensitivity of the Adh gene to DNase I are shown in Figure 3.

Figure 2. DNase I Digestion Profiles of the 5'U Region of the Adh Gene.
Nuclei isolated from leaves and nuclei isolated from cultured cells were digested with increasing concentrations of DNase I (0.00 to 0.80 μg/mL), as indicated at the top of the lanes.
(Top) EcoRI-digested DNA hybridized to the probe designed to end label indirectly the 5'U region (5'U in Figure 1).
(Bottom) Ethidium bromide stain of the gels shown at top. Lane M at right contains genomic DNA digested with EcoRV (RV), Xbal (X), HindIII (H), and Sall (S), and the positions of the sites are given relative to the start of transcription. Open triangles at bottom indicate the lanes selected to analyze the influence of the transcriptional state on the sensitivity to DNase I of the 5'U region. Scans of these lanes are shown in Figure 4. These same lanes are shown in Figure 3 for the analysis of the 3'D and 3'DD regions, and the same samples were used for the 5'UD region.

Figure 3. DNase I Digestion Profiles of the 5'UD, 3'D, and 3'DD Regions of the Adh Gene.
The blots used to study the influence of the transcriptional state on the sensitivity of the 5'U region to DNase I shown in Figure 2 were stripped and rehybridized with the probes designed to end label the 3'D and 3'DD regions. The lanes indicated with triangles in Figure 2 are shown here. To study the 5'UD region, the samples from the lanes indicated by triangles in Figure 2 were digested with EcoRV and hybridized with the probe designed to study the 5'UD region. L, leaves; CC, cultured cells; M, markers; RI, EcoRI; RV, EcoRV; X, Xbal; Sc, Sall.

4. Again, the DNA between approximately positions +1 and -500 is shown to be hypersensitive to DNase I in cells expressing the Adh gene. This hypersensitive area actually may extend somewhat 5' of position -500, because the sample selected to analyze the sensitivity in cultured cells had been extensively digested within the 5'U region. Thus, the DNA molecules in this sample could have been cut more than once in the 5'U region, and, because the cuts are detected by the end-labeling technique, the DNA located farther away from the end of the fragment to which the probe hybridized could appear less sensitive than it actually was.

Comparison of scans of the 3'D region shows that the coding region is generally more sensitive to DNase I in cells expressing the Adh gene than in cells where it is not expressed. This DNase I-sensitive area finishes around the end of transcription. In addition, comparison of the scans for the 5'UD and 3'DD regions indicates that they exhibit similar overall sensitivity to DNase I, regardless of the transcriptional state of the gene. Thus, the entire region of enhanced DNase I sensitivity in the transcriptionally active gene is demarcated by approximately position -500 on the 5' side and the end of transcription on the 3' side. Although the sensitive area could extend up to the border of the 5'U region (position -847), it clearly does not expand to the 5'UD region (between positions -847 and -2000).

Nucleosomal Arrays within the Transcriptionally Active Adh Gene
The 5'U and 3'D regions of the Adh gene showed enhanced sensitivity to DNase I in cells where the gene was expressed. To determine whether this sensitivity enhancement was
Figure 4. Influence of the Transcriptional State on the Sensitivity to DNase I of the Adh Gene.

Leaves and cultured cell samples digested with DNase I and hybridized with the probes designed to study the sensitivity of the 5'UD, 5'U, 3'D, and 3'DD regions of the Adh gene were selected as indicated in the text (lanes indicated with open triangles at the bottom of Figure 2 and all lanes shown in Figure 3), scanned, and plotted. To normalize the total amount of DNA, the scans were modified proportionally to make their areas equal. The thicker lines correspond to cultured cell samples, where the Adh gene is expressed, and the thinner lines correspond to leaf samples. The positions of some restriction sites (and their positions relative to the transcription start site) are indicated, as are the end and the start of transcription. H, HaeIII; Rl, EcoRI; RV, EcoRV; S, Sall; Sc, SacI; X, Xbal.

Figure 5. Nucleosomal Arrays within and around the Active Adh Gene.

DNA probes from the Adh gene. Figure 5 shows the resulting autoradiograms after sequentially hybridizing the same membrane with 10 different probes. Probe I was used as a control and was synthesized from a randomly cloned sequence 294 bp long (M.A. Vega-Palas and R.J. Ferl, unpublished results). This probe displayed a typical nucleosome ladder of DNA fragments corresponding to mononucleosomal and polynucleosomal particles, and it served as a critical control for the general integrity and distribution of nucleosomes in the chromatin of cells expressing Adh. Thus, several nucleosomes in a normal array (up to four) could be detected on this membrane. The positions corresponding to monosomic and polysomic particles are indicated by the small rectangles in Figure 5.

Hybridization of the membrane with the probes located between −964 and +1590 rendered different patterns. First, probes II (from −964 to −847) and V (from −451 to −385) did not display DNA fragments corresponding to nucleosomal particles. Indeed, the DNA regions that hybridize with these probes were not cut by micrococcal nuclease.

DNA from cultured cell nuclei digested with increasing concentrations of micrococcal nuclease (0.00 to 12.00 units/mL) was purified and resolved in agarose gels. The DNA was then transferred to a nylon membrane sequentially hybridized with the different probes represented on the restriction map. The positions of fragments corresponding to single, double, triple, and quadruple nucleosome particles are indicated by small rectangles. These nucleosome position markers are repeated for each segment to give a constant comparative reference for each hybridization of the blot. Restriction enzyme abbreviations are as given in the legend to Figure 4.
probes were significantly degraded even when micrococcal nuclease was not added. At no concentration of micrococcal nuclease was any hint of nucleosomes detected, because all of the hybridizing fragments were subnucleosomal in size. Second, probes III (from -847 to -671) and IV (from -558 to -451) clearly displayed the 146-bp mononucleosomal fragment as well as a fragment corresponding to dinucleosomal particles. Neither probe III nor probe IV detected DNA fragments corresponding to nucleosomal particles containing more than two nucleosomes, even at lower micrococcal nuclease concentrations. Third, probes VI (-451 to -273) and VII (-287 to -129) displayed the 146-bp fragment corresponding to the nucleosome core particle. However, DNA fragments associated with polynucleosomal particles were not detected with these probes. Fourth, probe VIII (-129 to -37) displayed a clear fragment corresponding to mononucleosomes but also faintly detected polynucleosomal particles. Fifth, probes IX (from -37 to +68) and X (from +1172 to +1590) displayed clear nucleosome ladders, similar in distribution to the control ladders seen with probe I, indicating that nucleosomes organized in a normal array were present within the coding region of the transcriptionally active Adh gene. The signal at the bottom of the blot when probe IX was used for hybridization was caused by RNA. The high molecular weight band detected with probe X at the top of the blot corresponds to plastid or mitochondrion DNA that shows some homology with probe X. Because the nucleases could not pass the membranes of either of these organelles, the plastid and mitochondrial DNAs were not digested. They were seen in agarose gels after staining with ethidium bromide as a high molecular weight band that was unaffected by nuclease concentration (data not shown).

To elucidate further the distribution of nucleosomes in the promoter, the sensitivity of the 5′U region to micrococcal nuclease was studied in naked DNA and in cultured cell nuclei by following the indirect end-labeling technique (Wu, 1980). Naked DNA and nuclei samples were digested with increasing concentrations of micrococcal nuclease, digested to completion with EcoRI, resolved in an agarose gel, and transferred to a nylon membrane. The membrane was then hybridized with the probe designed for end labeling was caused by RNA. Naked DNA and nuclei samples were digested with increasing concentrations of micrococcal nuclease, digested to completion with EcoRI, resolved in an agarose gel, and transferred to a nylon membrane. The membrane was then hybridized with the probe designed to end label the 5′U region (see Figure 1). The resulting digestion profiles of samples selected on the basis of light but equal digestion of DNA are shown in Figure 6. The center lane shows naked DNA, the lane at left shows DNA from cultured cells, and the lane at right shows the markers used for mapping. The digestion profile of the naked DNA sample indicates that the sequence preference of micrococcal nuclease within the 5′U region is noticeable (see lane N). Comparison of the lanes indicates that although micrococcal nuclease shares some sequence specificity with the Adh promoter, clear regions of enhancement and protection exist (Figure 6). This cutting pattern in nuclei from cells is most easily explained by assuming the presence of nucleosomes at fairly specific positions in the 5′U region of the Adh gene.

Figure 7 presents a model for the distribution of nucleosomes in the active Adh gene, based on correlations among the data in Figures 5 and 6. There is a nucleosome that is translationally phased just downstream of -30. This nucleosome is followed by a normal nucleosome array that continues throughout the coding region, because the transcription start region is protected against micrococcal nuclease, as shown in Figure 5, and two different probes from the coding region are able to display normal nucleosome ladders. These results are consistent with other data showing that the DNA region immediately downstream of the Adh transcription start site is protected against endonucleases in cells expressing the gene (M.A. Vega-Palas and R.J. Ferl, submitted manuscript).

The regulatory region contains specific nucleosome arrangements. We know that two nucleosomes are positioned between -500 and -800 because mononucleosomal and dinucleosomal particles of normal size are displayed when the nucleosome ladder is hybridized with probes III and IV, and probes II and V fail to detect nucleosomes at all. In addition, we know that a nucleosome is present in the promoter region somewhere between -130 and -350, because clear borders hypersensitive to micrococcal nuclease digestion are detected at these positions (Figure 6) and probes VI and VII detect only fragments corresponding to mononucleosomes (Figure 5). This nucleosome also may be localized downstream of -130, near -30, because probe VIII is clearly able to detect mononucleosome as well as a faint nucleosome ladder. However, given the intensity of the micrococcal nuclease–hypersensitive band.
Figure 7. Chromatin Structure of the Active Adh Gene.

This proposed model for the distribution of nucleosomes within the DNase I-sensitive domain of the Arabidopsis Adh gene summarizes the current data. At top is an interpretation of the distribution of nucleosomes based on the hybridization of specific probes to the nucleosome ladders of Figure 5; inverted triangles indicate the micrococcal nuclease data (MNase cleavages) given in Figure 6. Within the model itself, nucleosomes are indicated by the solid gray circles and are positioned according to the best fit of the data compilations at top. The loose positioning of the promoter area nucleosome is indicated by the best fit centered at -230; alternate locations for lightly shaded nucleosomes are also indicated. The range of the DNase I-sensitive domain and the limits of the DNase I hypersensitive region found in the 5'U region are indicated below the model.

The presence of this promoter region nucleosome is notable because it is not part of a typical nucleosome array. First, it is contained in a region of altered chromatin structure that shows DNase I hypersensitivity (see Figures 2 and 4). Second, the internucleosome DNA segments between this nucleosome and adjacent arrays are much longer than the linker DNA segments in bulk chromatin and show enhanced sensitivity to micrococcal nuclease, especially on the 5' side, where probe V detects no nucleosomes at all.

DISCUSSION

The Adh Gene as a Small DNase I-Sensitive Domain

Eukaryotic genes show preferential sensitivity to DNase I when they are expressed, and this is believed to reflect an open conformational state of chromatin (Weintraub and Groudine, 1976; Bellard et al., 1980; Stalder et al., 1980; Storb et al., 1981; Lawson et al., 1982; Wood and Felsenfeld, 1982; Murray and Kennard, 1984). The extent of this generalized sensitivity is ~24 kb for the chicken lysozyme gene (Jantzen et al., 1986), 33 kb for the chicken β-globin genes (Stalder et al., 1980; Hebbes et al., 1994), 100 kb for the chicken ovalbumin gene (Lawson et al., 1982), and 47 kb for the human apolipoprotein B gene (Levy-Wilson and Fortier, 1989). This sensitivity always seems to extend beyond the genes themselves, and in the case of the chicken β-globins, expands ~7 to 10 kb 5' upstream of β' and 3' downstream of β' (Stalder et al., 1980; Hebbes et al., 1994).

We have shown that the preferential sensitivity to DNase I of the active Adh gene is demarcated by approximately position -500 on the 5' side and the end of the mRNA on the 3' side (Figure 7). Current data indicate that all cis-acting elements probably are restricted to the first few hundred bases of the 5' flanking region (Ferl and Laughner, 1989; Dolferus et al., 1994). Little is known about termination of transcription at the 3' end of the Adh gene; however, the preferential sensitivity does not extend much beyond the recognized end of the mRNA to the surrounding sequences. Therefore, the conventionally accepted 5' and 3' boundaries of the Adh gene, defined by the coding and regulatory regions, define a small DNase I-sensitive domain. The extent of the domain is 2.5 kb, considerably shorter than other chromatin domains previously reported (Stalder et al., 1980; Lawson et al., 1982; Jantzen et al., 1986; Levy-Wilson and Fortier, 1989; Hebbes et al., 1994).

The biochemical reasons for the generalized sensitivity to DNase I of transcriptionally active chromatin are currently unknown, although several specific characteristics of this
chromatin have been described (van Holde et al., 1992; Hebbes et al., 1994). It is also unclear what defines the boundaries of the DNase I−sensitive domains. In the cases of the chicken lysozyme and human apolipoprotein B genes, SARs have been found to map with the boundaries of the sensitive regions and, therefore, could function in defining the sensitive domains (Phi-Van and Strätling, 1988). In addition, a SAR is located just upstream of maize Adh1 (Avramova and Bennetzen, 1993; Paul and Ferl, 1993). We are currently investigating the distribution of SARs around the Arabidopsis Adh gene to establish whether SARs similarly define the boundaries of its sensitive domain.

Nucleosomes in the Regulatory Region of the Active Adh Gene

The open conformational state of transcriptionally active chromatin is associated with disruption of nucleosomes in the regulatory and coding regions of genes. Nucleosome disruption in the regulatory regions has been reported previously in yeast and animal systems (Kornberg and Lorch, 1991; Felsenfeld, 1992; van Holde et al., 1992; Adams and Workman, 1993; Paranjape et al., 1994; Wolffle, 1994). In the case of the PHOS gene from yeast, for example, four nucleosomes located between −700 and +1 are disrupted upon transcriptional activation. Simultaneously, this region becomes hypersensitive to DNase I and restriction endonucleases (Almer and Hörz, 1986; Almer et al., 1986). The 5' upstream region of the active Adh gene between approximately −500 and +1 is hypersensitive to DNase I and restriction endonucleases; M.A. Vega-Palas and R.J. Ferl, submitted manuscript). However, this hypersensitivity does not reflect total depletion of nucleosomes. Instead, it is associated with the loose positioning and altered spacing of a nucleosome within a large gap. As shown in Figure 7, a typical nucleosome array begins near the start of transcription and extends 3; apparently throughout the coding region. In the upstream region, a pair of nucleosomes appears to be positioned between −500 and −800. The region of the promoter that is hypersensitive to DNase I exists between the nucleosome array of the coding region and the positioned nucleosome pair.

Specific Array of Nucleosomes in the Regulatory Region of the Adh Gene Coexists with Binding of Transcription Factors

Several sequences that bind nuclear proteins have been identified in the 5' upstream region of the active Adh gene. One of the sequences, referred to as the 4C box, is located between −145 and −148 and is a part of the anaerobic response element (Ferl and Nick, 1987; Walker et al., 1987; Dolferus et al., 1994). Three other sequences, from −312 to −308, from −218 to −210, and from −195 to −172, contain G-box−like elements, which are found in many eukaryotic genes with different regulatory patterns (Chodosh et al., 1989; Ferl and Laughner, 1989; Donald et al., 1990; Williams et al., 1992). According to our model, these cis−acting elements are located within the context of the loosely positioned nucleosome (Figure 7). In cases where cis−acting elements are associated with a nucleosome, they are usually wrapped around the nucleosome in a way that allows proper interaction of the transcriptional regulatory factors. The 4C box, home of the anaerobic response elements, is located near one of the main boundaries of this nucleosome. In addition, we have detected protein−DNA interactions in the DNA segment between approximately positions −450 and −350; this segment is nucleosome free (M.A. Vega-Palas and R.J. Ferl, unpublished results). Thus, nuclear proteins interact with DNA sequences wrapped around a nucleosome as well as with DNA located within a large internucleosome segment in the regulatory region of the active Adh gene.

The binding of some transcription factors to specific sequences in the regulatory region of the Adh gene could contribute to the specific arrangement of nucleosomes shown in Figure 7. In turn, this specific arrangement of nucleosomes could facilitate the binding of other transcription factors. Nuclear proteins that disrupt chromatin structure at the promoter region of genes have been described previously; GAGA is one such transcription factor from Drosophila that, like the G-box binding factor, binds to the promoter of many genes. Extending the analogy, it is interesting that the binding of GAGA to four sites in the heat shock hsp70 promoter causes nucleosome disruption and the acquisition of DNase I hypersensitivity (Tsukiyama et al., 1994).

Nucleosomes within the Coding Region of the Active Adh Gene

Nucleosomes are barely detected in the coding region of certain active genes (Cartwright and Elgin, 1984; Conconi and Clarence, 1993) but are well documented in others (van Holde et al., 1992). The coding region of the active Adh gene is clearly associated with nucleosomes, as shown by hybridization of nucleosome ladders with probes IX and X (Figure 5). Different models have been proposed to explain how nucleosomes can be present in the coding region of genes that are actively transcribed. These models predict that the transcription elongation process involves the displacement or disruption of nucleosomes by RNA polymerase (Adams and Workman, 1993).

In this study, we show that the Adh gene constitutes a small DNase I−sensitive domain defined by the boundaries of the Adh transcriptional unit, including the coding and regulatory regions. We have definitively determined that there is a remarkably close correspondence between a sensitive domain and a eukaryotic transcriptional unit. Previously reported DNase I sensitive domains include large regions of DNA that are not transcribed. In light of current and previous data, several general questions arise. Are other Arabidopsis transcriptional units organized as small DNase I−sensitive domains? Is the close correspondence between transcriptional unit and
chromatin domain unique in Arabidopsis, or does it generally exist in plants? Or, are eukaryotic genomes organized in long DNase I–sensitive domains composed of more-sensitive subdomains, reflecting different levels of chromatin organization? Our current efforts are directed toward examining larger areas of Arabidopsis chromatin.

We also show that a special nucleosome arrangement exists in the 5′ flanking region of the active Adh gene and that the nucleosomes within the coding region are displaced rather than released by the RNA polymerase. Our studies indicate that complex interactions among DNA, nucleosomes, and transcription factors influence gene expression in plants and that plant systems can provide illuminating data for comparison with animal and yeast systems.

METHODS

Plant Material and Culture Conditions

Plants (Arabidopsis thaliana ecotype Columbia) were grown in potting soil at 23°C under continuous illumination. Nuclei were isolated from leaves of 3-week-old plants. Arabidopsis cell suspensions (Ferl and Laughner, 1989) were maintained in the dark on a rotating shaker at 23°C using a 6-day subculture cycle. Nuclei were isolated from cells of 3- to 4-day-old cultures.

Nuclei Isolation, Digestion with Nucleases, and DNA Purification

Nuclear preparation and digestion with nucleases were performed using the modification of Paul et al. (1987). Approximately 10 g of leaves or cultured cells was collected and ground in a cold mortar with 5 mL of ice-cold nuclei isolation buffer (NIB; 50 mM Tris, pH 8.0, 5 mM MgCl₂, 15 mM NaCl, 1 mM β-mercaptoethanol, 0.1 mM phenylmethylsulfonyl fluoride [PMSF], 0.1 mM EGTA, 0.3 M sucrose). After the addition of 35 mL of NIB to a total of 4 mL per g of tissue, the resulting slurry was transferred to a motor-driven homogenizer (Wheaton Grind Potter-Elvehjem tissue grinder; Eberbach Corp., Ann Arbor, MI). After four passes with the pestle at full speed (400 rpm), the homogenate was filtered through four layers of cheesecloth and one layer of Miracloth (Calbiochem) into an ice-cold centrifuge tube. The nuclei were pelleted by centrifugation at 5600g at 4°C for 5 min, resuspended in 30 mL of NIB, and centrifuged again at 5600g at 4°C for 5 min. The wash with NIB was repeated.

For micrococcal nuclease digestion, the crude nuclei preparations were resuspended in 3 to 4 mL of micrococcal nuclease buffer (50 mM Tris, pH 8.0, 5 mM MgCl₂, 15 mM NaCl, 0.1 mM CaCl₂, 0.005 mM β-mercaptoethanol, 0.3 M sucrose) and separated into aliquots of 500 μL. The aliquots were preincubated at 37°C for 2 min, and 10 μL of DNase I previously diluted in DNase I dilution buffer (20 mM sodium acetate, 5 mM CaCl₂, 0.1 mM PMSF, 50% glycerol) was added. The samples were incubated at 30°C for 9 min, and the nuclei were collected by centrifugation in a microcentrifuge for 15 sec and resuspended in nuclei extraction buffer (NEB; 100 mM Tris, pH 8.0, 50 mM EDTA, 500 mM NaCl, 10 mM β-mercaptoethanol) (Paul et al., 1987) to stop the reaction.

Visualization of the Results

In general, results were visualized by the end-labeling technique (Wu, 1980) using probes (described in Figure 1) cloned into pSPT19 (Boehringer Mannheim) or pT7T3a (Gibco BRL) and produced by riboprobe synthesis using a commercial kit (Boehringer Mannheim) and 800 Ci/mmol UTP (Amersham). DNA fragments separated in agarose gels were transferred to nylon membranes (Hybond N+; Amersham, and hybridization was performed according to the manufacturer's protocol. In Figure 5, the DNA samples digested with micrococcal nuclease were not digested with a restriction enzyme before they were transferred to the membranes. Thus, a nucleosome ladder was displayed after the DNA fragments were resolved in agarose gels and stained with ethidium bromide.

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