The Plant Cell, Vol. 7, 1667–1680, October 1995

Matrix Attachment Regions and Transcribed Sequences within a Long Chromosomal Continuum Containing Maize *Adh1*

Zoya Avramova,[1] Phillip SanMiguel, Elena Georgieva,[2] and Jeffrey L. Bennetzen

Department of Biological Sciences, Purdue University, West Lafayette, Indiana 47907

We provide evidence for the location of matrix attachment sites along a contiguous region of 280 kb on maize chromosome 1. We define nine potential loops that vary in length from 6 kb to >75 kb. The distribution of the different classes of DNA within this continuum with respect to the predicted structural loops reveals an interesting correlation: the long stretches of mixed classes of highly repetitive DNAs are often segregated into topologically sequestered units, whereas low-copy-number DNAs (including the *alcohol dehydrogenase1* [*adh1*] gene) are positioned in separate loops. Contrary to expectations, several classes of highly repeated elements with representatives in this region were found to be transcribed, and some of these exhibited tissue-specific patterns of expression.

INTRODUCTION

The linear chromosomes of eukaryotes are compacted into a tiny organelle, the nucleus, in a manner that supports the biological functions of DNA with high fidelity, despite its enormous concentration. Different levels of DNA compaction exist, including the folding of the nucleosomal fiber into loops of different sizes. The bases of these loops are attached to a protein structure called the matrix or scaffold, and specific DNA sequences are responsible for the attachment of the chromatin to this structure (for reviews, see Gasser and Laemmli, 1987; Jackson, 1991). These loops might act not only as compaction units but also may define a separately regulated unit (for reviews, see Gross and Garrard, 1987; Bodnar, 1988; Goldman, 1988). Different experimental approaches have provided evidence that many biological activities are associated with the nuclear matrix; matrix attachment regions (MARs), beyond acting as anchoring sites, may bear positional information required for controlling transcription, may act as origins of replication, and may serve as chromatin border elements (Gasser, 1988; Gasser et al., 1989; Garrard, 1990; Bonifer et al., 1991; Eissenberg and Elgin, 1991).

Most of the data on MARs are in relation to known genes and their immediate surroundings; as a whole, information on domain organization and chromosome folding at a supragenic level is very limited. All of the current information on the relationship between loop organization, positioning of transcribed units, and potential origins of replication on a larger DNA continuum comes from two regions in Drosophila: a 320-kb continuum in the *rosy-Ace* region (Mirkovitch et al., 1988) and an 800-kb portion of chromosome 1 (Brun et al., 1990; Surdej et al., 1990). In addition, the 240-kb amplicon of the dihydrofolate reductase (DHFR) gene (Dijkwell and Hamlin, 1988), 200 kb around the mouse heavy chain IgH locus (Cockerill, 1990), and 90 kb containing the human globin loci (Jarman and Higgs, 1988) provide data on the relative distribution of MARs and transcribed units in relatively long genomic regions. In these latter cases, the genomic regions represent known loci containing several genes belonging to the same family and expressed in a developmentally controlled manner. Therefore, we still know very little about the structural and functional organization of large regions containing single genes or extensive genomic regions with no identified function. In plants, information on the loop organization of genomes is limited to the identification of matrix binding sites flanking known genes (Hall et al., 1991; Slatter et al., 1991; Breyne et al., 1992; Avramova and Bennetzen, 1993; Paul and Ferl, 1993; van der Geest et al., 1994).

An important aspect of the organization of most higher eukaryotic genomes is that the gene-containing component of the genome composes only a small fraction of the total DNA; the rest represents mainly repeated DNA. In maize, for example, the repetitive DNA makes up ~80% of the total nuclear genome (Flavell et al., 1974; Hake and Walbot, 1980). We still have little understanding of the possible roles repetitive DNA might serve, and we have only a limited knowledge of its structural organization within the maize genome.

A detailed analysis of a yeast artificial chromosome (YAC) containing the maize *alcohol dehydrogenase1* (*adh1*) locus has been published by Springer et al. (1994). They found the

[1] To whom correspondence should be addressed.
[2] Current address: Institute of Genetics, Bulgarian Academy of Sciences, Sofia, 1113 Bulgaria.

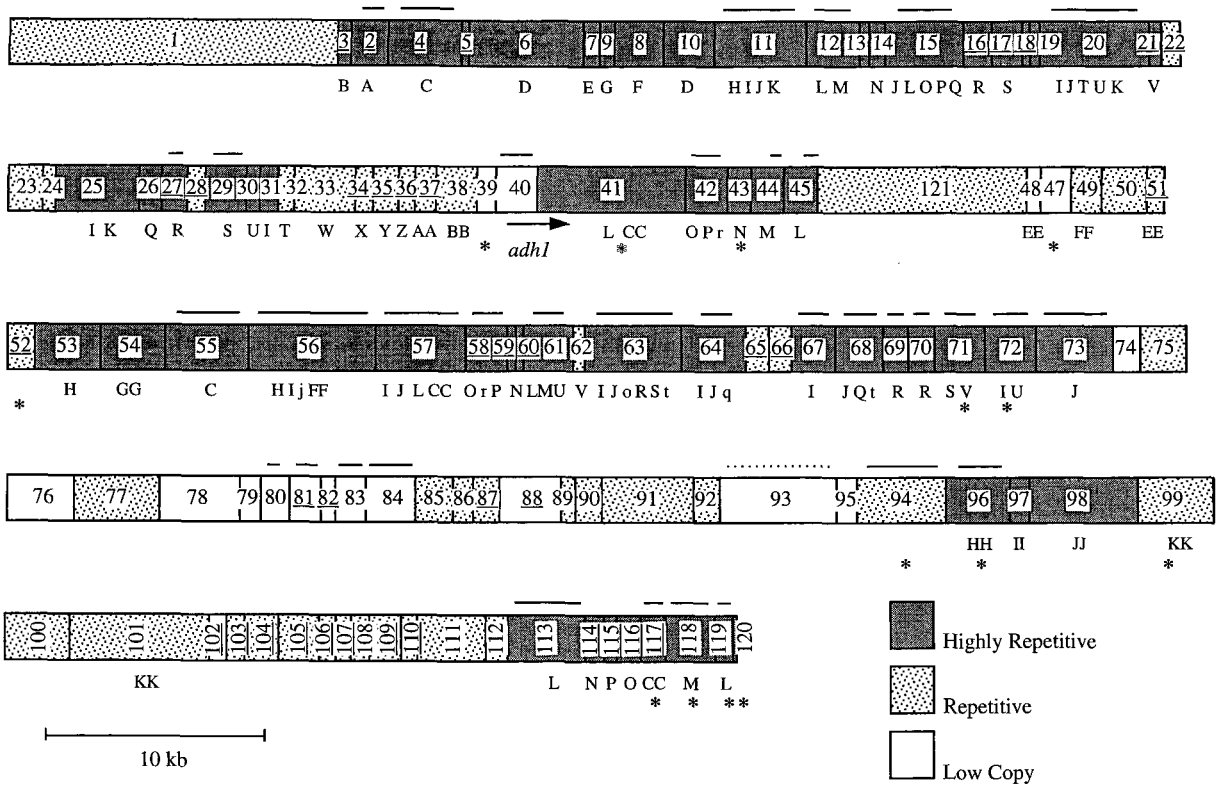

Figure 1. Map of 280 kb of DNA Flanking Maize *Adh1*.

Individual fragments obtained after triple digestion with XbaI, XhoI, and Bsu36I are numbered. Adjacent underlined numbers indicate fragments whose relative orders are not known. The darkly shaded, dotted, and open boxes designate fragments that are referred to as highly repetitive, repetitive, or low-copy-number (one to four bands on genomic DNA gel blot hybridization) DNAs, respectively. The letters below the boxes indicate a particular class of repeat present on the fragment. These repeat classes were previously defined by cross-hybridizational criteria, using each of the fragments containing highly repetitive DNA as a probe to filters containing the contiguous series of fragments from this region (Springer et al., 1994). The arrow under boxes 40 and 41 shows the orientation and size of the *adh1* transcript. The asterisks are below fragments on which MAR activity was detected. The open asterisk under fragment 41 indicates a weak MAR. The solid lines above the boxes indicate those fragments that hybridized with RNA in nuclear run-on analyses. The dotted line above box 93 indicates that this fragment hybridizes weakly with RNA from nuclear run-on assays, but RNA gel blot hybridizations did not detect a transcript. Fragment 46, with MAR activity, is not shown due to our inability to position it relative to fragments 42 to 45. The ordering and repetitive DNA characterizations shown in this figure come from Springer et al. (1994), with some additional subsequent characterizations.

contiguous 280-kb region contained mostly repetitive DNA of mixed classes in long blocks, which are separated by short blocks of single-copy DNA. As is generally true in the maize genome, modified highly repeated DNAs and unmodified low-copy DNAs were found in separate domains (Bennetzen et al., 1994). These studies prompted us to look for a possible relationship between the linear and spatial organization of the maize genome. One approach to gain insight into such a relationship is to study the distribution of the different classes of DNA sequences with respect to the putative structural domains into which a particular chromosomal region may be folded. A second step would be to determine the possible arrangement of functional units (transcribed regions, replicons, and recombination sites) within the structural domains.

Earlier, a MAR adjacent to various regulatory elements was identified 5′ to the *adh1* gene (Avramova and Bennetzen, 1993; Paul and Ferl, 1993). Now, we report the identification and characterization of a MAR downstream of the gene, closing *adh1* into a putative structural loop of ~12 kb. In addition, we screened ~280 kb of DNA around *adh1* for sites capable of binding specifically to the nuclear matrix. A number of DNA fragments containing MAR elements were localized, dividing the region into putative loops of heterogeneous sizes. The DNA fragments harboring the MARs were found to contain different reiteration classes of sequences. Occasionally, two MARs were found located on adjacent fragments carrying dispersed repeats, and four MARs were clustered at the 3′ end of the YAC clone.

The whole region was screened for transcribed units by DNA hybridization to labeled RNA synthesized in nuclear run-on experiments. Nuclei from anaerobically stressed roots, mature green leaves, and tassels were used to characterize the tissue

specificity of the transcribed elements. Unexpectedly, many of the dispersed highly repetitive sequences were found to be transcribed. Although most of the repetitive elements were expressed in all three tissues, some exhibited a tissue-specific pattern of expression, as did some low-copy-number sequences.

RESULTS

Localization of the 3′ *adh1* MAR

The idea that a gene might be located in a structural loop bracketed by two MARs has received considerable support from studies on a number of animal genes. The available data suggest that some MARs might serve as insulators, shielding the promoter and other regulatory elements from the effect of the surrounding chromatin (for reviews, see Bonifer et al., 1991; Eissenberg and Elgin, 1991). Recently, van der Geest (1994) reported the first case of a plant gene bordered by two attachment elements and showed that the two flanking MARs exert a shielding effect on a reporter gene in transgenic plants.

Earlier, we had demonstrated that isolated nuclear matrices from maize leaves specifically bind MAR sequences of both plant and animal origin (Avramova and Bennetzen, 1993). We also localized a MAR 5′ to the transcribed region of *adh1*. Using gel blot hybridization analysis of restriction nuclease–digested nuclear haloes, Paul and Ferl (1993) identified the same MAR, providing confirmation of the validity of both approaches for identifying MARs in the maize genome.

In a previous characterization of the *adh1* region (Springer et al., 1994), a clone has been isolated that contains sequences flanking the gene (Figure 1, fragments 39 through 45). This clone (λ170) was investigated for a possible MAR site downstream of the gene. Due to the abundance of highly repetitive DNA immediately flanking the coding sequence (Figure 1), the only possible approach to detect a matrix binding region is to screen for fragments with the capacity to bind specifically to isolated nuclear matrices. The preferential binding of an end-labeled restriction fragment, in the presence of others covering neighboring regions and in the presence of a large excess of unlabeled DNA, would serve as an indication of MAR activity. Strong MARs were arbitrarily defined as those that bound >20% of input DNA to purified matrix; a range of 20 to 50% was seen for these strong MARs. Weak MARs were defined as those that bound 2 to 10% of input DNA to the purified matrix. We considered it necessary to make a distinction between strong MARs and weak MARs because they may have different functions (Bodnar, 1988; Surdej et al., 1990).

As shown in Figure 2A, two of the input fragments covering the genomic region from 39 to 45 show prominent binding to the matrix. They were identified as fragments 43 and 39. The latter contains the MAR 5′ to the *adh1* gene identified earlier (Avramova and Bennetzen, 1993; Paul and Ferl, 1993). The location of fragment 43 is downstream of the gene-coding region (Figures 1 and 2C) in an area rich in repetitive DNA.

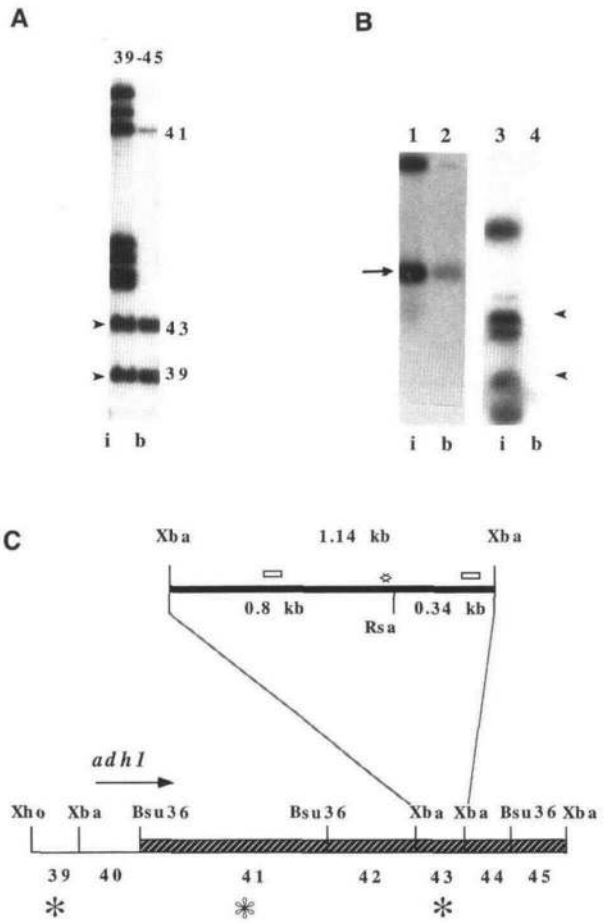

Figure 2. Binding of Fragments near *Adh1* to Maize Nuclear Matrices.

(A) Fragments generated by digestion of λ170 (covering the genomic region between fragments 39 to 45) with three restriction nucleases (XbaI, XhoI, and Bsu36I). The left lane (i) illustrates the input end-labeled fragments, and the adjacent lane (b) shows the preferential binding (indicated by arrowheads) of fragments 39, 41, and 43 (shown at right). i, input fragments; b, matrix bound fragments.

(B) Digestion of pX1.14 with XbaI, generating the maize insert (arrow) and the vector (lane 1). Preferential binding of the 1.14-kb maize fragment to the nuclear matrix is shown in lane 2. Digestion of the same plasmid with both XbaI and RsaI produced several fragments (shown in lane 3), two of which originated from the maize insert (arrowheads at right). Lane 4 shows that cleavage of the 1.14-kb XbaI fragment at the RsaI site abolishes the capacity of the maize DNA to bind to the matrix.

(C) Schematic presentation of the region analyzed, containing the *adh1* gene. Fragment numbers correspond to those in Figure 1. The asterisks mark the MAR-containing fragments; the open asterisk designates the presence of a weaker MAR on fragment 41. Its precise position on the 7-kb fragment was not determined. The open boxes above indicate the location of the two sequences homologous with the Drosophila topoisomerase II consensus motif. The ⨳ sign designates the position of the BUR relative to the RsaI site. The arrow indicates the length, orientation, and position of the *adh1* transcript. Filled boxes indicate highly repetitive DNA.

Fragments 43 and 39 would bracket the gene in a putative loop of ~12 kb.

An additional MAR was found downstream of the gene and closer to the *adh1* coding region (Figure 2A, fragment 41). Although more weakly bound by the matrix preparation, this DNA displays a reproducible affinity that was visible even at high concentration of competitor DNA and was also detected as a weak MAR with matrices purified using the lithium diiodosalicylate (LIS) approach (data not shown). This MAR is located on a 7-kb fragment that contains at least two different classes of repetitive elements and some low-copy-number DNA (Bennetzen et al., 1994; Springer et al., 1994). Its potential binding to the nuclear matrix would sequester the *adh1* gene into a miniloop of 3 to 9 kb, depending on the exact location of the MAR (Figure 2C). Although the possible involvement of this weak MAR in the structural organization and expression of *adh1* is not excluded, in this study we concentrated on the more prominent 3′ MAR.

Characterization of a Strong MAR 3′ to *adh1*

Fragment 43 was isolated from λ170 and subcloned into plasmid pCEL80 (Lee et al., 1987) to generate pX1.14. Digestion of the latter with XbaI released the entire insert (Figure 2B, arrow at lane 1) and the vector. In the presence of competitor DNA, only the 1.14-kb insert bound preferentially to isolated nuclear matrices (Figure 2B, lane 2). When pX1.14 was cut with XbaI and RsaI, several fragments were generated (Figure 2B, lane 3). The arrowheads mark the position of the 800- and 340-bp fragments resulting from digestion of the insert (Figure 2C). Neither of the resulting fragments retained the capacity to bind to the nuclear matrix (Figure 2B, lane 4), indicating that splitting the 1.14-kb fragment at the RsaI site destroys its MAR activity. This is in contrast to the earlier observation of the 5′ MAR, when digestion of the 750-bp MAR region with ApoI resulted in two equal-sized fragments that were both able to bind to the matrix (Avramova and Bennetzen, 1993).

The 1.14-kb MAR-containing fragment was sequenced via γδ transposon mutagenesis (Strathmann et al., 1991); the resulting sequence has GenBank accession number U29136. The 1.14-kb fragment is 70% AT rich and contains two elements homologous with the topoisomerase II consensus sequence (allowing one mismatch). These sequences are found at positions 332 to 350 and 1032 to 1052. A putative base unpairing region (BUR) was identified at positions 760 to 770, immediately upstream of the RsaI site at position 801. The BUR sequence, AATATATT, matches perfectly an element shown to be essential for matrix binding (Kohwi and Kohwi-Shigematsu, 1990; Bode et al., 1992). The sequence features contained in the 1.14-kb fragment include AT richness, a topoisomerase II consensus sequence, and a BUR; these are considered indispensable for MAR activity. It is believed that the specific structural conformation of the DNA rather than the primary sequence is responsible for MAR activity.

Screening for MARs Downstream of the *adh1* Loop

Previous studies in our laboratory have determined that the region around the *adh1* gene is rich in highly repetitive sequences, representing different classes of dispersed repeats (Springer et al., 1994). These repeats are found scattered throughout the genome in thousands of copies whose functions, if any, are largely unknown. Therefore, it was of major interest to study the possible spatial folding of a genomic region composed mainly of dispersed repeats, particularly because data concerning the loop organization of large repetitive blocks are practically nonexistent.

The available data on the loop-related structure of larger chromosomal regions have been derived from studies of nuclear halo preparations digested with restriction nucleases. Gel blot hybridization analysis of soluble and insoluble (matrix bound) DNA fractions to probes covering large genomic regions would identify all MARs of that particular region in a single step. This is how the large regions in the Drosophila genome (Mirkovitch et al., 1988; Brun et al., 1990; Surdej et al., 1990) and 240 kb around the mouse DHFR gene (Dijkwell and Hamlin, 1988) were screened. Although straightforward, such an approach is inapplicable to genomic regions rich in repetitive elements. An alternative approach is to test each individual clone for the ability of sequences to bind specifically to exogenously added nuclear matrices.

Figure 3 shows the results from the MAR screening of a number of λ clones covering a contiguous region of ~280 kb around the maize *adh1* locus. Individual clones, or sometimes two neighboring λ clones covering overlapping regions on the chromosome, were digested with three restriction nucleases (XbaI, XhoI, and Bsu36I) that do not cut within the vector (Springer et al., 1994). All but the largest fragments obtained as a result of this triple digestion originated from the inserted maize DNA only. The two bands at the top of each lane represent the λ arms plus a segment of adjacent maize DNA. Fragments were end labeled and tested for their ability to bind to isolated nuclear matrices in the presence of competitor DNA. In Figure 3A, the results of the screening of the entire region downstream of the *adh1* gene are presented. The first lane in Figure 3A contains the restriction fragments of a clone partially overlapping λ170. The presence of fragment 43 in this clone and its binding to the matrix serve as an internal positive control, marking an attachment site of the adjacent loop downstream of the gene. All input fragments (lanes i) were identified, and those binding to the matrix (lanes b) are numbered to the right of the relevant lanes.

A smaller fragment, number 46, was run off of the gel in this experiment but was detected to have matrix binding activity in subsequent experiments (Figure 3A). However, because there are several small fragments in this region similar in size to fragment 46, we have not been able to determine its exact location on the contiguous map. Hence, this fragment and its putative MAR are not included in Figure 1. Depending on its precise location, the MAR on fragment 46 could be twinned

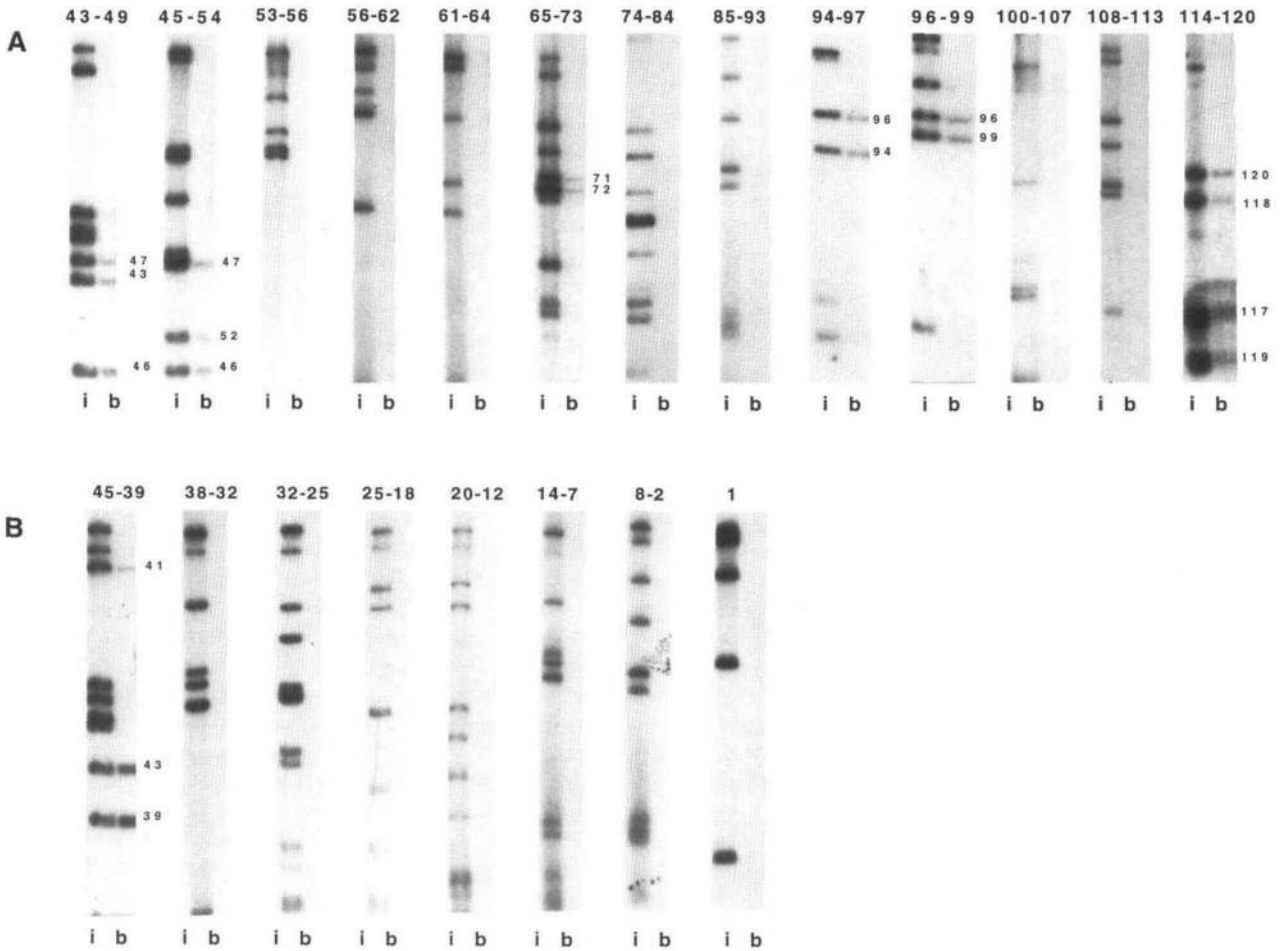

Figure 3. Screening of Sequences Flanking *Adh1* for Matrix Attachment Regions.

(A) Screening of the region downstream of *adh1*. The numbers on top indicate the end fragments of each genomic region screened for MARs. Lanes i contain the input fragments obtained as a result of the triple digestion of the respective region with the nucleases XbaI, XhoI, and Bsu36I. The adjacent lanes b show the fragments bound by the matrix in the presence of 100 μg/mL of *E. coli* DNA. Numbers at right indicate the designations of fragments with MAR activity. Because the vector arms do not contain sites for XbaI, XhoI, or Bsu36I, the two highest molecular weight bands in each lane consist of the λ arms plus fragments of vector-adjacent maize DNA.
(B) Screening of the region upstream of *adh1*. Designations are as given in **(A)**.

with fragment 43 to serve as a single MAR, as we observed for the MAR 5′ to *adh1* (Avramova and Bennetzen, 1993), or could act with fragment 43 to determine a miniloop of 7 kb or less containing some subset of fragments 42 to 46.

Downstream of the 3′ MARs bordering *adh1*, an attachment site was discovered on fragment 47. The putative loop that could be enclosed by MARs on fragments 43 and 47 would contain several intermixed classes of repetitive DNAs but would also have low-copy-number DNAs on fragments 48 and 47 at its *adh1* distal end. This putative loop would be ~14 kb in length. The next predicted loop downstream, bounded by MARs on fragments 47 and 52, would be ~6 to 7 kb in length and would contain primarily (or exclusively) repetitive DNAs.

Continuing downstream, the next region with affinity to the nuclear matrix is found at a distance of ~42 kb (Figure 3A, fragments 71 and 72). These two adjacent MARs are weak (~5 and ~3.5% of input DNA bound, respectively) and would close a relatively long loop composed primarily of highly repetitive DNA. It is possible that the pair of attachment sites ensures a better framing and segregation of this block of repeats from the adjacent region. The next MAR (fragment 94) is ~45 kb downstream, serving as a possible boundary to a region composed mainly of different low-copy-number and middle repetitive sequences (Figure 1). A second MAR is located a short distance farther downstream (Figure 3A, fragment 96). The precise location of the attachment sites on these fragments

is not known, and therefore, these MARs could define a loop of anywhere from 0 bp (if attachment sites are adjacent at the boundary between fragments 96 and 97) to 7 kb (if MARs are located at the distal ends of their respective fragments). The next putative loop would also be small (MAR on fragment 99) and mainly encompasses a highly repetitive *gypsy*-like retroelement we have named *Cinful* (Bennetzen, 1995). The most *adh1* distal region contains primarily middle repetitive elements intermixed with low-copy-number sequences. Its distal end is bordered by a cluster of different classes of highly repetitive DNA that contains several MARs (Figure 3A, fragments 117 to 120).

Summarizing these results, it appears that this chromosomal subregion can potentially be subdivided into at least eight loops of very heterogeneous sizes. The matrix binding sites come in pairs at two locations and as a cluster of four at the distal 3′ end of the chromosome. A similar observation was made in the heavy chain IgH locus of mouse, where several MARs have been found clustered on adjacent fragments carrying dispersed repeats (Cockerill, 1990).

Screening for MARs 5′ to *adh1*

Figure 3B shows the results of our screening for matrix binding sites in the subregion upstream of *adh1*. Clone λ170, containing the gene and the two flanking MARs (fragments 39 to 45), was used as a positive control for MAR activity. In contrast with the situation downstream of the gene, no matrix attachment site was found upstream of the putative loop containing *adh1*. This subregion is composed mainly of highly reiterated DNAs, occasionally interspersed with middle repetitive elements.

The *adh1* distal end of this subregion contains ~20 kb of mostly middle repetitive DNA that has no sites for the three restriction enzymes (XbaI, XhoI, and Bsu36I) used in this study. The three insert fragments shown in the last lane i of Figure 3B were generated by MluI digestion. None of them showed any potential to bind the matrix, thus leaving a loop of >75 kb open. This is the largest predicted loop in the genomic region mapped, with its 5′ end beyond the limits of available probes.

Identification of Transcribed Units in a 280-kb Region Flanking *adh1*

The mapping of MARs along the contiguous region surrounding the *adh1* gene in maize suggested a pattern for its possible folding into separate structural units—loops that are fastened at their ends by matrix attachment sites. A question of basic importance is the relationship between such a structural loop and its possible biological activity. One way to approach this question is to screen the region for the presence and distribution of transcribed units.

As a first step in this direction, we probed, for hybridization to nuclear RNA, the overlapping contiguous series of clones in the *adh1* region. λ clones were digested with XbaI, XhoI, and Bsu36I, fractionated, blotted, and hybridized with nuclear RNA labeled by run-on synthesis in isolated nuclei (see Methods). As in the MAR analyses, these digestions always yielded two high molecular weight fragments that contained the λ arms plus some vector-adjacent maize DNA not characterized in the fingerprinting of these clones. Hence, hybridization of RNA to these largest bands, apparently to the maize DNA attached to the vector arms, was ignored in this analysis. RNA from nuclei of different tissues was used as a probe to investigate the tissue specificity of any identified transcripts.

Starting from the 3′ end of the YAC, λ clones covering ~120 kb upstream (fragments 120 to 63) were selected, digested with the three restriction enzymes, and fractionated in a 1% agarose gel (Figure 4, lanes 1 to 10). After blotting, this filter was successively hybridized with labeled nuclear RNA prepared from leaves, anaerobic roots, and tassels (Figure 4B). The second subregion investigated is composed of the chromosome upstream of fragment 74. This long (~190 kb) stretch of DNA is composed mainly of highly repetitive sequences with occasional middle repetitive or low-copy-number elements. Digested λ clones covering the subregion are shown in lanes 8 to 10 and 8 to 18 of Figure 4. The two filters overlap at the middle repetitive zone covered by fragment 77. The upstream end of the subregion, corresponding to the *adh1* distal end of the YAC, is occupied by ~20 kb of middle repetitive DNA. It did not hybridize to any of these three RNAs preparations (data not shown) and was not analyzed further.

The fragments hybridizing with the nuclear run-on RNA were identified and are schematically shown at center in Figure 4A. Lanes 1 of Figure 4B illustrate hybridization in all three tissues to highly repeated DNAs localized at the 3′ end of the YAC. The adjacent region, containing middle repetitive blocks of sequences with interspersed low-copy-number regions (lanes 2 and 3), does not exhibit detectable hybridization. In Figure 4B, lane 4, two bands identified as middle repetitive fragment 94 and highly repetitive fragment 96 are found to hybridize to RNA from leaf nuclei only.

The weak band in Figure 4B, lane 5, hybridized only to leaf RNA preparations and was identified as fragment 93. At this point, however, it is not clear whether this low-copy-number fragment contains a gene, because additional studies failed to identify its corresponding message (see below). A few bands in lane 6 (Figure 4B), identified as fragments 84, 83, 81, and 80 (the last two have the same mobility), were found to hybridize with leaf and root RNAs. The lack of a signal from the corresponding bands in tassels suggests specific expression in roots and leaves. Its putative message was identified by RNA gel blot hybridization (see below).

To our knowledge, all of the ~75 kb 5′ to *adh1* is repetitive (Bennetzen et al., 1994; Springer et al., 1994). Hybridization to RNA is shown in Figure 4B (lanes 8 to 10) and Figure 4C (lanes 8 to 18). Analysis of the hybridizing fragments revealed that, along with the *adh1* gene (marked by arrowheads in lanes 15), several highly repetitive sequences are transcribed (for their identification, see the center of Figure 4A). The specific

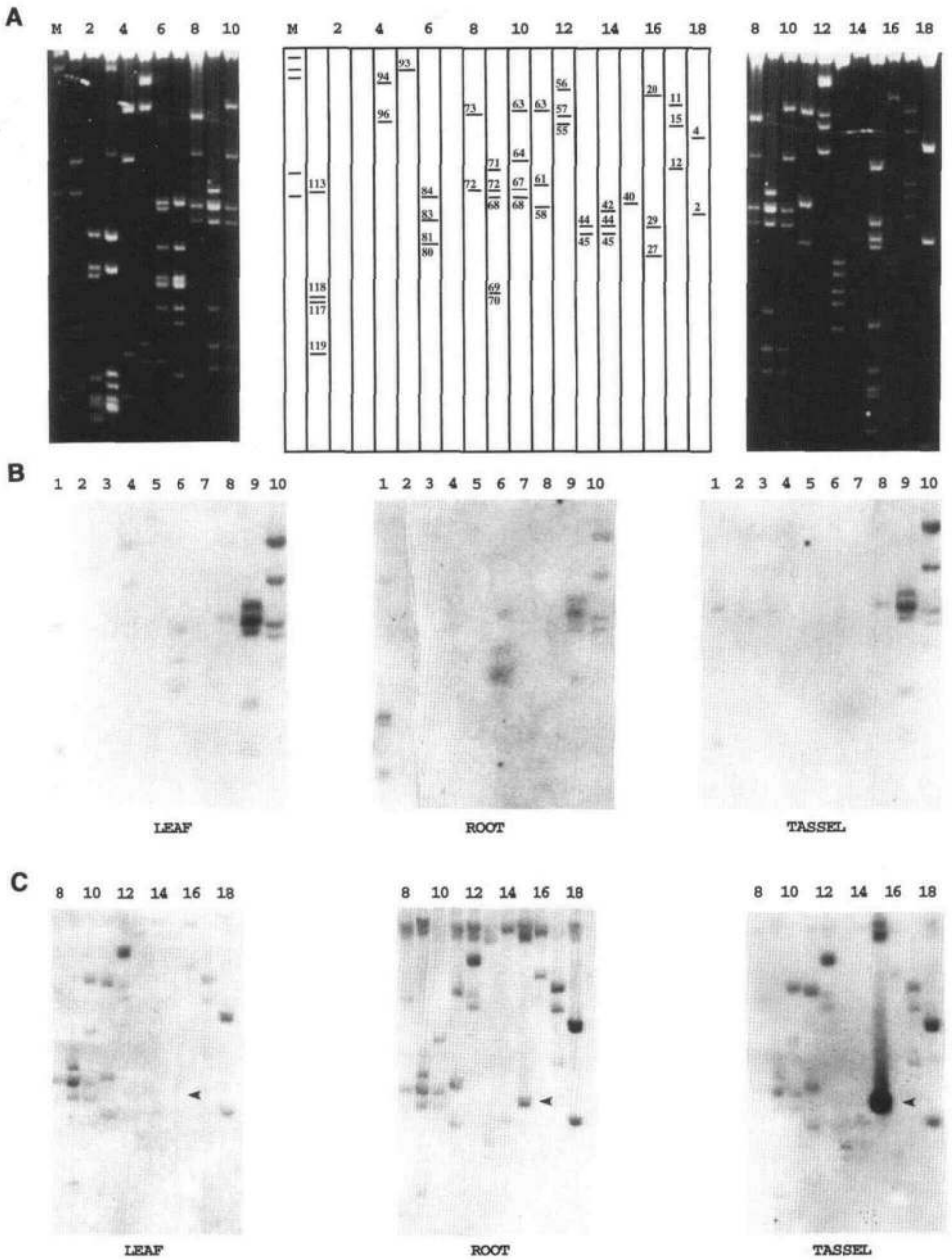

Figure 4. Hybridization of Nuclear Run-On RNA to Sequences in the *Adh1* Region of the Maize Genome.

(A) Ethidium bromide–stained gels of λ clones covering the 280-kb *adh1* region after digestion with XbaI, XhoI, and Bsu36I. The contiguous region has been divided into two subregions, and the two gels overlap the region between fragments 77 to 63 (lanes 8 to 10). At center is a schematic presentation of the fragments hybridizing to RNA as visualized in **(B)** and **(C)**; they are numbered as given in Figure 1. Stained molecular length markers are shown in lane M.

(B) Screening the region downstream of *adh1* for transcribed units. A filter replica of the gel depicted at left in **(A)** was hybridized to ^{32}P-labeled RNA generated by run-on synthesis in leaf, root, and tassel nuclei, respectively. The same filter was stripped after each hybridization and rehybridized with a different RNA preparation. Lanes 1 to 10 contain fragments 120 through 62.

(C) Screening for transcribed sequences upstream of *adh1*. A filter replica of the gel depicted at right in **(A)** was hybridized with nuclear run-on RNA. The clones on this filter cover the maize genomic region between fragments 77 and 2. Lane 10 artifactually fails to show hybridization of root run-on RNA to fragment 63 (center), although it was detected in a previous analysis (see lane 10 in **[B]**). We also used fragment 63 in RNA gel blot analysis of root RNA, and it exhibits intense hybridization (data not shown). The arrowheads indicate fragment 40 in lanes 15. Fragment 40 contains the coding sequence of the *adh1* gene. For identification of the hybridizing fragments, see the text and the schematic presentation in **(A)**.

Table 1. Expression of DNA Fragments Flanking the Maize *Adh1* Gene

Fragment	Copy No.[a]	Root[b]	Leaf[b]	Tassel[b]
2	HR	+ +	+ +	+ +
4	HR	+	+ +	+ +
11	HR	+	+	+
12	HR	+	+	+
15	HR	+	+	+
20	HR	+	+	+
27	HR	+ −	−	+ −
29	HR	+ −	−	+
40	SC	+	−	+ +
42	HR	+ −	+ − −	+ −
44	HR	+ −	+ − −	+ −
45	HR	+ −	+ − −	+ −
55	HR	+	+	+
56	HR	+ +	+ +	+ +
57	HR	+ +	+ +	+ +
58	HR	+	+	+
61	HR	+ +	+ +	+ +
63	HR	+ +	+ +	+ +
64	HR	+	+	+
67	HR	+	+	+
68	HR	+	+	+
69	HR	+	−	+
70	HR	+	−	+
71	HR	+ +	+ +	+
72	HR	+ +	+ +	+
73	HR	+	+	+
80	SC	+	+	−
81	SC	+	+	−
83	SC	+	+	−
84	SC	+	+	−
93	SC	−	+ −	−
94	MR	−	+	−
96	HR	−	+	−
113	HR	+	+	+
117	HR	+	+	+
118	HR	+	+	+
119	HR	+	+	+

[a] HR, highly repetitive DNA; SC, single-copy DNA; MR, middle repetitive DNA.

[b] (−) indicates no expression; (+ −) indicates a low level of expression; (+ − −) indicates a very low level of expression; (+) indicates a medium level of expression; (+ +) indicates a high level of expression.

hybridizations of fragment 40, containing *adh1*, in roots and tassels and its absence from leaves are in accordance with the known tissue-specific expression of the gene (Freeling, 1973; Woodman and Freeling, 1981).

Some repetitive sequences were found to be expressed to a different degree in the three types of nuclei studied. The visible intensities of hybridization, presumed to reflect the level of expression of the various units present in the region, are summarized in Table 1. The different expression patterns of some classes of repeats, in addition to the complete lack of expression by others, argue for tissue-specific transcription of some classes of these dispersed and highly repetitive DNAs. The most intensely hybridizing bands correspond to fragments composed of mixed classes of interspersed repeats, making it impossible at this stage to sort out the individual contribution by any particular class.

Analysis of Transcribed Units by RNA Gel Blot Hybridization

RNAs isolated from anaerobic roots and green leaves were size fractionated and hybridized to various labeled DNA probes. Lanes 1 in Figure 5 show the fractionation pattern of total RNA preparations from anaerobic roots and green leaves. Hybridization of RNA from anaerobic roots to a labeled plasmid clone containing the *adh1* gene resulted in one major band (Figure 5A, lane 2) corresponding to the expected transcript (Dennis et al., 1984). This band was not observed when leaf RNA was probed with the same DNA (Figure 5B, lane 2), in full agreement with the known tissue-specific expression of the gene.

When a λ clone spanning the low-copy region defined by fragments 78 to 84 was used as a hybridization probe for root and leaf RNAs (Figure 5, lanes 3), an RNA transcript of 2800 bases was identified in both preparations. Rehybridization of the same filters with a mixture of two isolated fragments from the 5′ end of the clone, fragments 78 and 79, failed to yield any hybridization, confirming that the expressed region is located within fragments 80 to 84.

Another low-copy-number fragment, 93, was found to hybridize weakly to leaf RNA in run-on analysis (see above). However, in RNA gel blot analyses, fragment 93 failed to hybridize detectably to RNA in either leaf or root preparations (data not shown).

When highly repetitive fragment 4 was used as a probe, heavy smears with a few distinct bands were observed on hybridization to both root and leaf RNAs (Figure 5, lanes 4). In lane 5 of Figure 5A, the RNA on the filter was subjected to alkaline hydrolysis before hybridization, demonstrating that the hybridizational pattern is due to an RNA–DNA interaction and not to contaminating genomic DNA. In lane 6 of Figure 5A, hybridization was performed with RNA fractionated and blotted under nondenaturing conditions so that only single-stranded nucleic acid molecules (that is, RNA) would be retained by the filters. These results confirm our observation that some classes of repeated DNA elements are heavily transcribed in maize nuclei.

The detected expression of some dispersed highly repetitive DNAs was somewhat unexpected, because it is commonly believed that these sequences are transcriptionally silent. Therefore, we asked whether these repeats were present in cDNA libraries. A seedling cDNA library was screened with DNA of fragment 4 as probe, and numerous positive colonies

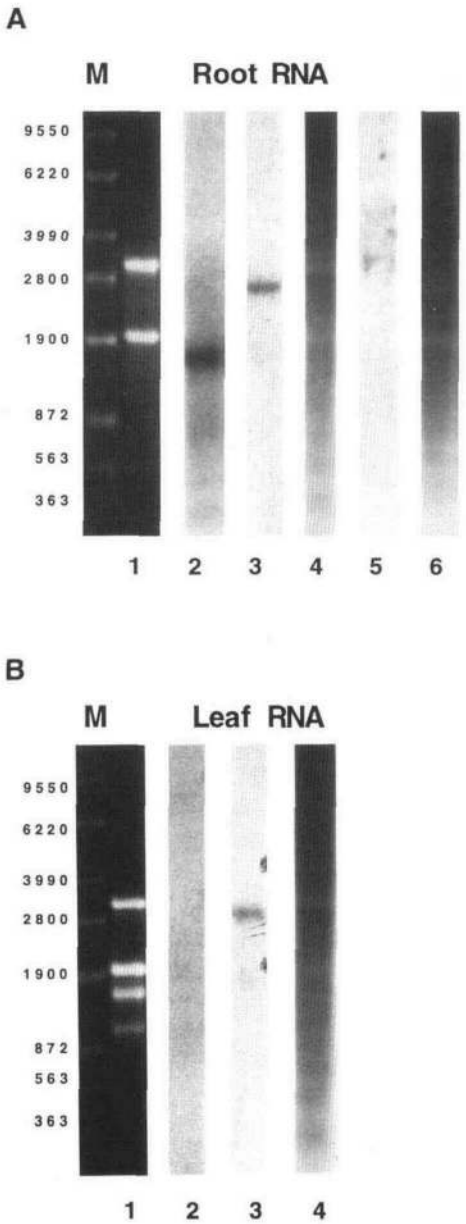

Figure 5. RNA Gel Blot Analysis of Fragments in the *Adh1* Continuum.

(A) RNAs were fractionated through 1% agarose gels. In lane M and lanes 1 to 5, the RNA preparations were denatured before and during electrophoresis. Depicted in lanes 1 and 2 are ethidium bromide–stained markers (lane M; the numbers at left show the number of bases of marker RNAs) and total root RNA (lane 1). Shown in lane 2 is hybridization of filter-replicated lane 1 RNA to a plasmid containing *adh1*. The size of the message (observed with anaerobic roots only) agrees with previous reports (Dennis et al., 1984). Shown in lane 3 is hybridization of the RNA-containing filter with the fragments 78 to 84, which detects a band of ~2.8 kb. Shown in lane 4 is a rehybridization of the filter from lane 3, after removal of the previous probe, with highly repetitive fragment 4, containing a class C repeat. Shown in lane 5 is hybridization with a fragment 4 probe to a gel blot filter containing root RNA that had been treated with 0.5 N NaOH prior to hybridization. Loss of hybridization indicates that the pattern observed in lane 4 is due to an RNA–DNA hybridization. Shown in lane 6 is hybridization of labeled fragment 4 DNA to a filter containing root RNA gel fractionated and blotted under nondenaturing conditions so that only single-stranded nucleic acid molecules would be transferred to the filter. **(B)** Size markers (lane M) and total leaf RNA (lanes 1 to 4) were fractionated through a denaturing 1% agarose gel. Other than the RNA source, the lanes shown are as described in **(A)**: lane M, ethidium bromide–stained markers; lane 1, ethidium bromide–stained total leaf RNA; lane 2, RNA gel blot hybridization to an *adh1* probe; lane 3, RNA gel blot hybridization to fragments 78 to 84; and lane 4, RNA gel blot hybridization to a fragment 4 probe.

were detected. Approximately a dozen clones were isolated, and inserts were found to be of various sizes. These inserts contain highly repetitive regions with good homology to various genetic units of retrotransposons (A. Tikhonov, P. SanMiguel, J.L. Bennetzen, and Z. Avramova, unpublished observations).

MARs, Transcripts, and the Junctions between Low-Copy-Number and Highly Repetitive DNA Domains

Previous analyses (Bennetzen et al., 1994; Springer et al., 1994; Z. Avramova and J.L. Bennetzen, unpublished observations) have now identified 17 low-copy-number fragments (numbers 39, 40, 47, 48, 74, 76, 78 to 84, 88, 93, 95, and 102) in the 280 kb flanking *adh1*. From a combination of DNA renaturation and gel blot hybridization analyses (Hake and Walbot, 1980; Bennetzen et al., 1994), it has been determined that in mature tissues, most of the nuclear DNA of maize is subdivided into two structurally distinct domains: an extensively cytosine-methylated domain containing highly repetitive DNAs, interspersed with middle repetitive sequences, and a relatively unmethylated domain consisting of low-copy-number DNAs (genes) intermixed with middle repetitive DNAs (Bennetzen et al., 1994). Identifying the boundaries between these domains is often not simple, however, because middle repetitive DNAs can be found in either domain type.

Near *adh1*, our detailed studies have identified fragments 39 and 41 as containing the junctions between long repetitive tracts and the 5′ and 3′ regions of the gene, respectively. These two fragments also exhibit MAR activity. In contrast, the two long (>75 and 42 kb) tracts composed primarily or exclusively of mixed highly repetitive and middle repetitive DNAs only contain identified MAR activity at or near their boundaries (Figure 6). In general, with some exceptions, the tracts of mixed low-copy-number and middle repetitive DNAs are found in separate putative loops, unlike mixtures of highly repetitive and middle repetitive DNAs (Figure 6).

Our RNA studies uncovered putative transcripts from three low-copy-number sequences, represented on fragments 40 to 41 (*adh1*), 80 to 84, and 93. In each case, these known (*adh1*) or putative genes are predicted to be uninterrupted by MARs and are found in loops that contain little detected highly repetitive DNA (Figure 6).

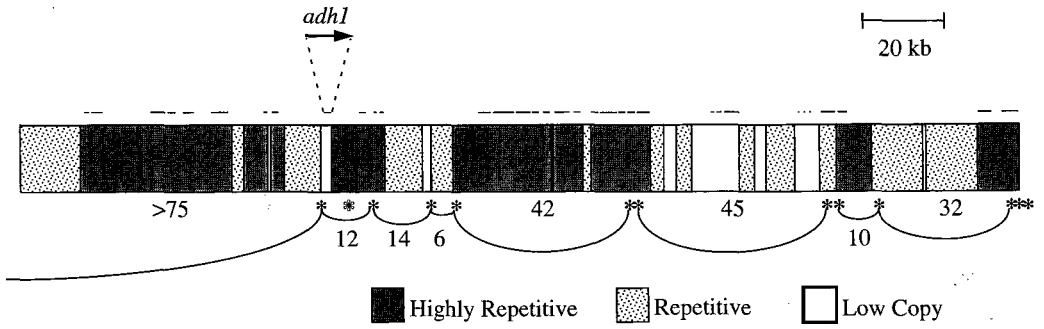

Figure 6. Putative Loops, Transcripts, and Repeats in 280 kb of DNA Flanking *Adh1*.

This is a simplified and condensed version of Figure 1, with similar symbols and added loops predicted by our analyses. Asterisks indicate the positions of identified MARs, with the open asterisk indicating a weak MAR found 3′ to *adh1*. Numbers indicate the predicted size, in kilobases, of these putative loops. Filled boxes indicate regions of highly repetitive DNA, and dotted boxes represent regions of less repetitive DNAs. The open boxes are tracts of single-copy- or very low-copy-number DNA. Lines above the boxes indicate the position and approximate size of regions in this continuum that hybridized to nuclear run-on transcripts. The arrow indicates the location and orientation of the *adh1* transcript.

DISCUSSION

Definition of the Borders of a Putative Structural Domain Bearing the *adh1* Gene

The segregation of a gene or a cluster of genes into structurally defined domains has been extensively investigated in animal systems (Gasser and Laemmli, 1986; Jarman and Higgs, 1988; Mirkovitch et al., 1988; Phi-Van and Stratling, 1988; Surdej et al., 1990), but this has only recently been reported for a plant gene (van der Geest et al., 1994). The importance of such information is contained in the possibility that the MARs flanking a gene might delimit an individually regulated loop (for reviews, see Bonifer et al., 1991; Eissenberg and Elgin, 1991).

By testing the capacity of the genomic fragments covering ~280 kb flanking the *adh1* gene to bind preferentially to isolated nuclear matrix, we identified a strong MAR ~10 kb downstream of the coding sequence. In this study, we discriminated between strong and weak MARs because it is possible that these different subclasses of MARs are involved in different functions. Strong MARs may delimit structural domains containing the transcriptional units (Surdej et al., 1990), and it has been shown that the subclass of matrix binding fragments able to support autonomous replication in yeast belongs to the category of strong MARs (Brun et al., 1990). Such relationships have not been found for weak MARs, and they can be located in introns and coding sequences (Cockerill and Garrard, 1986; Kas and Chasin, 1987; Brun et al., 1990; Cockerill, 1990; Avramova and Paneva, 1992; Romig et. al., 1994). The possibility, however, that weak MARs might be involved in the dynamic folding of a loop into subdomains in response to regulatory signals is intriguing and remains to be explored. The weak MAR (2 to 3% of input fragment bound to the isolated matrix) on fragment 41 would define a loop of <10 kb containing the *adh1* gene almost exclusively.

The MAR-bearing sites on fragments 39 and 43 are defined as strong (~50% of the input fragment bound to isolated matrix). Fragment 43 contains highly repetitive DNA defined as a member of class N by cross-hybridizational criteria (Springer et al., 1994). Other fragments along this same genomic region contain class N repeats but do not bind to the matrix preparations (Figure 1). This observation is in agreement with earlier reports suggesting that, although matrix binding activity occasionally resides within a repeat, no particular dispersed repeats routinely act as MARs (Cockerill, 1990; Surdej et al., 1990). Alternatively, the matrix binding capacity of fragment 43 may be due to an element located within the repeat that is not present in the other N-containing fragments. Fragment 43 is the longest of the N-containing fragments in this continuum, and removal of 340 bp from this fragment by RsaI cleavage abolishes MAR activity. Hence, an essential component of MAR function, at or flanking this RsaI site, is located near a putative BUR. According to our current understanding, the capacity of a DNA region to open the double helix under superhelical strain is crucial for MAR activity (Kohwi and Kohwi-Shigematsu, 1990; Bode et al., 1992), and it is possible that the adjacent sequences are necessary for BUR function.

The presence of a motif matching the consensus element for topoisomerase II is often taken as one feature required for MAR activity. Topoisomerase II has been identified as a component of the nuclear matrix and the chromosomal scaffold (Berrios et al., 1985; Earnshaw and Heck, 1985). The presence of sequence elements in MARs similar to the cutting sites preferred by the enzyme has been widely used as an argument for the probable involvement of topoisomerase II in interactions with the MARs (for reviews, see Gasser and Laemmli, 1987; Gross and Garrard, 1987). This feature, however, became controversial after the discovery of different consensus sequences for topoisomerase II utilized in vivo and in vitro (Kas and Laemmli, 1992) and the evidence that the

consensus might differ substantially between vertebrates and Drosophila (Spitzner and Muller, 1988). Nevertheless, the presence of sequence motifs in MARs homologous with the Drosophila topoisomerase II consensus (Cockerill and Garrard, 1986) might still be important as an indication of a specific DNA conformation contained in this motif that is required for MAR activity.

Distribution of MARs across a Large Chromosomal Region in Maize

Models involving location and function of MARs may be biased by a data set restricted mainly to known genes. Therefore, it is important to gather more information about the structural organization in a region of the genome encompassing more than a single gene. We have chosen a chromosomal region containing one known gene, *adh1*, embedded in various reiteration classes of DNA. Previous studies have indicated that >50% of the different classes of highly repetitive DNA in maize have representatives in this 280-kb region (Springer et al., 1994). We suggest therefore that the *adh1* chromosomal region can legitimately represent the maize genome, as has been suggested by previous more general analyses of maize single-copy and repetitive blocks (Hake and Walbot, 1980; Bennetzen et al., 1994). The availability of a large number of overlapping λ clones covering this genomic region has made a detailed MAR analysis possible.

The distribution of identified MARs suggests the possible folding of this chromosomal region into loops of very heterogeneous sizes. In other species, the distance between the centers of two consecutive MARs (putative loop size) has been seen to vary from 4 to 200 kb (for reviews, see Gasser and Laemmli, 1987; Gasser, 1988). The first putative loop identified in plant chromatin is 3.3 kb (van der Geest et al., 1994). Small loop sizes, 3 to 15 kb, were indicated along 90 kb of the human globin gene complex, and it has been suggested that MARs may cluster in the immediate vicinities of genes to form miniloops (Jarman and Higgs, 1988).

In maize, we identified one predicted loop of >75 kb upstream of the *adh1* gene and numerous loops (ranging in length from 6 to 45 kb) downstream. It is not clear what relationship may exist between loop size and transcriptional activity. Some data suggest that highly expressed genes reside in smaller loops, whereas multiple genes with lower activity are present in one bigger loop (Mirkovitch et al., 1988). However, no relationship was found between transcriptional activity and loop size along a contiguous 800 kb of Drosophila chromosome 1 (Surdej et al., 1990), and loss of activity has been reported when the loop size of a transgenic neomycin phosphotransferase II gene was decreased (Mlynárová et al., 1994).

Transcribed Units within the *adh1* Region

Because we know little about the possible existence of genes in the vicinity of *adh1*, we decided to search for transcribed units. Hybridization of nuclear run-on RNA to subclones of the maize YAC identified three transcribed single-copy regions: one belonging to the *adh1* gene, another spanning ~8 kb of genomic DNA (fragments 80 to 84), and one contained in a 7-kb fragment (93). Subsequent RNA gel blot hybridizations with fragments 80 through 84 identified a potential mRNA of ~2800 bases in both leaf and root preparations. These results argue that this region contains a gene. A corresponding message for fragment 93 was not revealed, which makes it impossible at this point to form firm conclusions about its expression. The tissue-specific pattern of expression and the expected size of the message observed for *adh1* agreed with expectations (Dennis et al., 1984) and support the validity of our results.

An unexpected observation was the hybridization of different classes of repeats to RNA from nuclear run-on experiments. Contrary to the general belief that most of the dispersed highly repetitive elements of maize are transcriptionally silent, our results indicate the opposite. Not all classes of repeated elements hybridized to RNA, and those that hybridized did so with very different intensities (Table 1). The strongest signal was usually seen with fragments containing various combinations of repeats, so it is not possible at this point to evaluate the individual input of any particular class of repeats. Hence, it is likely that some of the repeats hybridize to more than one type of RNA, which could account for some of the differences in hybridizational intensity. For instance, using fragment 4 as a probe, numerous positive colonies were found in a cDNA library. Five clones were analyzed; four were found to represent overlapping clones of one class of highly repeated sequences and one was a cDNA for a different highly repeated element.

Data on the location of transcribed elements within structural loops at the supragenic level are limited to very few studies, and these suggest that attachment to the matrix and transcription are not systematically associated (Mirkovitch et al., 1988; Surdej et al., 1990). It has been proposed that strong MARs may delimit one or more transcriptional units, serve as origins of replication, or both (Brun et al., 1990; Surdej et al., 1990). We found that the *adh1* gene appears to be located within a loop delimited by two strong MARs that, potentially, can be subdivided into two smaller loops by a weak MAR downstream of the coding region. The 3′ end of the larger (12-kb) predicted *adh1* loop is occupied by repetitive DNA, a fraction of which (fragment 42) may also be transcribed. The expression of this repetitive element may be seen in anaerobic roots and tassels and is barely detectable in leaves, a tissue-specificity pattern similar to that for *adh1* expression. However, we have not yet determined whether a particular repetitive DNA located in the *adh1* region is actually transcribed or (more likely) whether the detected expression actually comes from a subset of the thousands of other copies dispersed throughout the genome.

Eight hybridizing fragments, representing 15 classes of identified repeats (Springer et al., 1994), are contained in the predicted >75-kb loop that is 5′ to *adh1*. In the putative 42-kb loop 3′ to *adh1*, 14 hybridizing fragments are found, mostly those classes of repeats also observed in the putative >75-kb

loop. In the adjacent 45-kb domain, composed predominantly of middle repetitive and single-copy sequences, only one transcribed region (fragments 80 to 84) was identified and confirmed. The putative 45-kb loop is separated at both ends from flanking highly repetitive blocks by pairs of MARs. These double MARs might provide a more efficient insulating effect to DNA within the predicted 45-kb loop, or may be due to digestion within a MAR that releases two functional units (Avramova and Bennetzen, 1993), or both. The putative 10- and 32-kb loops near the end of the contiguous series exhibited hybridization to run-on RNA only in repetitive DNAs.

MARs at the Boundaries between Highly Repetitive Blocks and Low-Copy-Number Sequences

The precise identification and mapping of MARs, different sequence elements, and transcribed units in a large region of contiguous eukaryotic DNA are not trivial undertakings. In our study, some of the MARs might have been missed due to cleavage within a MAR by one of the three restriction enzymes used in our fingerprinting of the *adh1* region. Low-copy-number sequences (for example, genes) would not have been detected if they were present on a fragment that also contained a significant percentage of repetitive DNA. For instance, fragment 41 was scored as highly repetitive by our DNA gel blot hybridization experiments (Springer et al., 1994), but we know that it is more than one third single-copy DNA by our more detailed analyses (Bennetzen et al., 1994). The only way to define precisely the components of these chromosomal regions is to sequence them entirely and compare the results with those obtained for related cDNAs and repeated elements isolated from other regions of the genome.

Hence, given the limitations of our approach, we were surprised to find a strong (but not perfect) correlation between MAR locations and the junctions of repetitive and low-copy-number DNA blocks. This adds support to the idea that MARs may act as insulating factors in maize to limit position effects. This result also suggests that the mapping of MARs across a long chromosomal continuum might be an effective approach to find where the genes are in that region.

In the *adh1* region, the long stretches of highly repetitive DNA seem to be segregated into units that are topologically sequestered from low-copy-number sequences (shown schematically in Figure 6). However, it will be necessary to study individual MAR-containing DNA in more detail to define more precisely the borders of each structural domain relative to these elements.

Defining the functional significance of MARs is a major challenge. Their involvement in folding the genome is just one of their potential roles. In the future, it will be interesting to test and compare their capacity to serve as "insulating" elements or as origins of replication. The identification of several MARs in a large characterized genomic region is a first step in our attempts to understand the relationship between genome structure and function in maize.

METHODS

Isolation of Nuclear Matrices and Identification of Matrix Attachment Regions

Functionally competent nuclear matrices from green leaves of maize (*Zea mays*) seedlings were prepared as described by Avramova and Bennetzen (1993). We have shown that, in our hands, high-salt and lithium diiodosalicylate (LIS) extraction procedures were equally efficient in yielding maize matrix preparations competent to recognize and specifically bind matrix attachment region (MAR)-containing DNA. In this study, we used high-salt extraction because we found it easier (less washes are needed) to remove traces of the extraction reagent from matrix-containing pellets. However, all weak MAR binding was confirmed with LIS-extracted matrices. MAR binding assays were carried out as previously described (Avramova and Bennetzen, 1993). Various amounts of competitor *Escherichia coli* DNA (from 10 to 200 μg/mL) were used in preliminary experiments to establish the optimal concentration under which only strong MARs would be identified. Strong MARs were defined on the basis of the relative amount (>20%) of input DNA bound to the matrix. Weak MARs were defined as those that bound 2 to 10% of input DNA.

λ Clones Used in This Study

More than 100 λ clones were subcloned and characterized from a yeast artificial chromosome (YAC) that contains ~280 kb of the maize genome (Springer, 1993; Springer et al., 1994). Clones were chosen so that the whole maize region would be covered, sometimes with large overlaps, and cut with XbaI, XhoI, and Bsu36I (New England Biolabs, Beverly, MA). The resulting fragments were 5′ end labeled with γ ^{32}P-ATP and T4 polynucleotide kinase and tested for the presence of MAR-containing sequences.

Nuclear Run-On Transcription Analysis

Nuclei were isolated from mature green leaves, tassels of 6-week-old plants, and anaerobically stressed roots as described by Steinmuller and Apel (1986). Anaerobic root nuclei were obtained after leaving whole seedlings (with 7 to 8 cm of primary roots) totally immersed in water under vacuum for 17 hr (Sachs et al., 1980). The nuclear run-on transcription analysis was essentially as described by Paul and Ferl (1991), with the following modifications. After 15 min run-on RNA synthesis in the presence of ^{32}P-UTP, 250 μg/mL of tRNA, 10 mM of $MgCl_2$, and 20 μg/mL DNaseI (Sigma) were added to the reaction mixture. After an additional 15 min at 30°C, 1% SDS, 5 mM EDTA, and 100 μg/mL of proteinase K were added. After 20 min at 37°C, this mixture was extracted with phenol-chloroform, followed by filtration through Sephadex G-50 (Sigma). The fraction containing the labeled RNA was immediately introduced to the prehybridized filter in a solution of 5 × SSC (1 × SSC is 0.15 M NaCl, 0.015 M sodium citrate), 0.5% SDS, 20 mM sodium phosphate buffer, pH 6.5, 20 mM EDTA, 100 mM Tris, pH 7.4, 2.5 × Denhardt's solution (1 × Denhardt's solution is 0.02% Ficoll, 0.02% PVP, 0.02% BSA), and 150 μg/mL of denatured salmon sperm DNA. Hybridization was performed for 40 hr at 65°C, followed by five to six washes with 2 × SSC, 0.5% SDS, at 65°C for a total of 3 to 4 hr.

Isolation of Total RNA and Hybridization

Total RNA was isolated according to the procedure of Chomczynski and Sacchi (1987), following the protocol and using the isolation system of Promega (technical bulletin No. 087). RNA markers also came from Promega. RNA was fractionated in 1% agarose–formaldehyde gels in 3-(*N*-morpholino)propanesulfonic acid buffer and blotted onto membranes (Micron Separations, Westborough, MA). Hybridization and washing conditions were as described above for run-on transcription analysis.

DNA Sequencing

The 1.14-kb DNA containing the MAR 3′ to alcohol dehydrogenase 1 (*adh1*) was characterized by γδ transposon–facilitated DNA sequencing (Strathmann et al., 1991). The 1.14-kb XbaI fragment was cloned into a pUC19 derivative, pCEL80 (Lee et al., 1987), conferring ampicillin resistance and propagated in *E. coli* DH5α F^+ containing the γδ transposon. The recipient cells were *E. coli* DH5α F^- Rif^+. Conjugation was performed for 6 hr at 37°C with shaking at 60 rpm, and the cells were plated onto Luria-Bertani agar containing 80 μg/mL of methicillin, 20 μg/mL of ampicillin, and 100 μg/mL of rifampicin and then grown overnight. From 10 picked colonies, seven carried a γδ transposon insertion in the 1.14-kb fragment and three were in the plasmid vector. After restriction nuclease analysis, three of these seven colonies were chosen and sequenced by the dideoxy method using primers homologous with the inverted termini of γδ.

ACKNOWLEDGMENTS

We are indebted to Young Kwan Jin (Purdue University) for subcloning the MAR-containing fragment 3′ to *adh1* into pCEL80. This work was supported by U.S. Department of Agriculture National Research Initiative Grant Nos. 93-37300-8769 (to Z.A.) and 94-37300-0299 (to J.L.B.). P. S.M. was supported by a National Science Foundation training grant for plant genetics.

Received April 5, 1995; accepted July 24, 1995.

REFERENCES

Avramova, Z., and Bennetzen, J.L. (1993). Isolation of matrices from maize leaf nuclei: Identification of a matrix-binding site adjacent to the *Adh1* gene. Plant Mol. Biol. **22,** 1135–1143.

Avramova, Z., and Paneva, E. (1992). Matrix attachment sites in the murine α-globin gene. Biochem. Biophys. Res. Comm. **182,** 78–85.

Bennetzen, J.L. (1995). The contributions of retroelements to plant genome structure, function, and evolution. Trends Microbiol., in press.

Bennetzen, J.L., Schrick, K., Springer, P.S., Brown, W.E., and SanMiguel, P. (1994). Active maize genes are unmodified and flanked by diverse classes of modified, highly repetitive DNA. Genome **37,** 565–576.

Berrios, M., Oscheroff, M., and Fisher, P. (1985). *In situ* localization of DNA topoisomerase II, a major polypeptide component of the *Drosophila* nuclear matrix fraction. Proc. Natl. Acad. Sci. USA **82,** 4142–4146.

Bode, J., Kohwi, Y., Dickinson, L., Joh, T., Klehr, D., Mielke, C., and Kohwi-Shigematsu, T. (1992). Biological significance of unwinding capacity of nuclear matrix-associating DNAs. Science **255,** 195–197.

Bodnar, J.W. (1988). A domain model for eukaryotic DNA organization: A molecular basis for cell differentiation and chromosome evolution. J. Theor. Biol. **132,** 479–507.

Bonifer, C., Hecht, A., Saueressig, H., Winter, D.M., and Sippel, A.E. (1991). Dynamic chromatin: The regulatory domain organization of eukaryotic gene loci. J. Cell. Biochem. **47,** 99–108.

Breyne, P., Van Montagu, M., Depicker, A., and Gheysen, G. (1992). Characterization of a plant scaffold attachment region in a DNA fragment that normalizes transgene expression in tobacco. Plant Cell **4,** 463–471.

Brun, C., Dang, Q., and Miassod, R. (1990). Studies of an 800 kilobase DNA stretch of the *Drosophila* X chromosome: Comapping of a subclass of scaffold-attached regions with sequences able to replicate autonomously in *Saccharomyces cerevisiae.* Mol. Cell. Biol. **10,** 5455–5463.

Chomczynski, P., and Sacchi, N. (1987). Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal. Biochem. **162,** 156–157.

Cockerill, P.N. (1990). Nuclear matrix attachment occurs in several regions of the IgH locus. Nucleic Acids Res. **18,** 2643–2648.

Cockerill, P.N., and Garrard, W.T. (1986). Chromosomal loop anchorage of the kappa immunoglobulin gene occurs next to the enhancer in a region containing topoisomerase II sites. Cell **44,** 273–282.

Dennis, E.S., Gerlach, W.L., Pryor, A.J., Bennetzen, J.L., Inglis, A., Llewellyn, D., Sachs, M.W., Ferl, R.J., and Peacock, W.J. (1984). Molecular analysis of the alcohol dehydrogenase (*Adh1*) gene of maize. Nucleic Acids Res. **12,** 3983–3995.

Dijkwell, P.A., and Hamlin, J.L. (1988). Matrix attachment regions are positioned near replication initiation sites, genes, and an interamplicon junction in the amplified dihydrofolate reductase domain of Chinese hamster ovary cells. Mol. Cell. Biol. **8,** 5398–5409.

Earnshaw, W.C., and Heck, M.M.S. (1985). Localization of topoisomerase II in mitotic chromosomes. J. Cell Biol. **100,** 1716–1725.

Eissenberg, J.C., and Elgin, S.C.R. (1991). Boundary functions in the control of gene expression. Trends Genet. **7,** 335–340.

Flavell, R.B., Bennett, M.D., Smith, J.B., and Smith, D.B. (1974). Genome size and the proportion of repeated nucleotide sequence DNA in plants. Biochem. Genet. **12,** 257–269.

Freeling, M. (1973). Simultaneous induction by anaerobiosis and 2,4-D of multiple enzymes specified by two unlinked genes: Differential *Adh1* and *Adh2* expression in maize. Mol. Gen. Genet. **127,** 215–227.

Garrard, W.T. (1990). Chromosomal loop organization in eukaryotic genomes. Nucleic Acids Mol. Biol. **4,** 163–175.

Gasser, S.M. (1988). Nuclear scaffold and higher-order folding of eukaryotic DNA. In Architecture of Eukaryotic Genes, G. Kahl, ed (Vienna: VCH Verlagsgeselschaft), pp. 461–471.

Gasser, S.M., and Laemmli, U.K. (1986). Cohabitation of scaffold binding regions with upstream/enhancer elements of three developmentally regulated genes of *D. melanogaster.* Cell **46,** 521–530.

Gasser, S.M., and Laemmli, U.K. (1987). A glimpse at chromosomal order. Trends Genet. **3,** 16–22.

Gasser, S.M., Amati, B.B., Cardenas, M.E., and Hofmann, J.-F. (1989). Studies on scaffold-attachment sites and their relation to genome function. Int. Rev. Cytol. **119,** 57–96.

Goldman, M.A. (1988). The chromatin domain as a unit of gene regulation. BioEssays **9,** 50–55.

Gross, D.S., and Garrard, W.T. (1987). Poising chromatin for transcription. Trends Biochem. Sci. **12,** 293–297.

Hake, S., and Walbot, V. (1980). The genome of *Zea mays*, its organization and homology to related grasses. Chromosoma **79,** 251–270.

Hall, G., Allen, G.C., Loer, D.S., Thomson, W.F., and Spiker, S. (1991). Nuclear scaffolds and scaffold-attachment regions in higher plants. Proc. Natl. Acad. Sci. USA **88,** 9320–9324.

Jackson, D.A. (1991). Structure–function relationships in eukaryotic nuclei. BioEssays **13,** 1–10.

Jarman, A.P., and Higgs, D.R. (1988). Nuclear scaffold attachment sites in the human globin gene complexes. EMBO J. **7,** 3337–3344. J. **12,** 1059–1065.

Kas, E., and Chasin, L.A. (1987). Anchorage of the Chinese hamster dihydrofolate reductase gene to the nuclear scaffold occurs in an intragenic region. J. Mol. Biol. **198,** 677–692.

Kas, E., and Laemmli, U.K. (1992). *In vivo* topoisomerase II cleavage of *Drosophila* histone and satellite III repeats: DNA sequence and structural characteristics. EMBO J. **11,** 705–716.

Kohwi, Y., and Kohwi-Shigematsu, T. (1990). Torsional stress stabilizes extended base-unpairing in supressor sites flanking heavy chain enhancer. Biochemistry **29,** 9551–9560.

Lee, L., Fenoll, C., and Bennetzen, J.L. (1987). Construction and homologous expression of a maize *Adh1* based *Nco*I cassette vector. Plant Physiol. **85,** 327–330.

Mirkovitch, J., Spiere, P., and Laemmli, U.K. (1988). Genes and loops in 320,000 base-pairs of the *Drosophila melanogaster* chromosome. J. Mol. Biol. **190,** 255–258.

Mlynárová, Ľ., Loonen, A., Heldens, J., Jansen, R.C., Keizer, P., Stiekema, W.J., and Nap, J.-P. (1994). Reduced position effect in mature transgenic plants conferred by the chicken lysozyme matrix-associated region. Plant Cell **6,** 417–426.

Paul, A.-L., and Ferl, R.J. (1991). In vivo footprinting reveals unique *cis*-elements and different modes of hypoxic induction in maize *Adh1* and *Adh2*. Plant Cell **3,** 159–168.

Paul, A.-L., and Ferl, R.J. (1993). Osmium tetroxide footprinting of a scaffold attachment region in the maize *Adh*1 promoter. Plant Mol. Biol. **22,** 1145–1151.

Phi-Van, L., and Stratling, W.H. (1988). The matrix-attachment region of the chicken lysozyme gene co-maps with the boundaries of the chromatin domain. EMBO J. **7,** 655–664.

Romig, H., Ruff, J., Fackelmayer, F.O., Patil, M.S., and Richter, A. (1994). Characterization of two intronic nuclear-matrix-attachment regions in the human topoisomerase I gene. Eur. J. Biochem. **221,** 441–449.

Sachs, M.M., Freeling, M., and Okimoto, R. (1980). The anaerobic proteins of maize. Cell **20,** 761–767.

Slatter, R.E., Dupree, P., and Gray, J.C. (1991). A scaffold-associated DNA region is located downstream of the pea plastocyanin gene. Plant Cell **3,** 1239–1250.

Spitzner, J.R., and Muller, M.T. (1988). A consensus sequence for cleavage by vertebrate DNA topoisomerase II. Nucleic Acids Res. **16,** 5533–5556.

Springer, P.S. (1993). Genomic Organization of *Zea mays* and Its Close Relatives. PhD Dissertation (West Lafayette, IN: Purdue University).

Springer, P.S., Edwards, K.J., and Bennetzen, J.L. (1994). DNA class organization on maize *Adh*1 yeast artificial chromosomes. Proc. Natl. Acad. Sci. USA **91,** 863–867.

Steinmuller, K., and Apel, K. (1986). A simple and efficient procedure for isolating plant chromatin which is suitable for studies of DNase I–sensitive domains and hypersensitive sites. Plant Mol. Biol. **7,** 87–94.

Strathmann, M., Hamilton, B.A., Mayeda, C.A., Simon, M.I., Meyerowitz, E.M., and Palazzolo, M.J. (1991). Transposon-facilitated DNA sequencing. Proc. Natl. Acad. Sci. USA **88,** 1247–1250.

Surdej, P., Got, C., Rosset, R., and Miassod, R. (1990). Supragenic loop organization: Mapping in Drosophila embryos of scaffold-associated regions on a 800 kilobase DNA continuum cloned from the 14B-15B first chromosome region. Nucleic Acids Res. **18,** 3713–3722.

van der Geest, A.H.M., Hall, G.E., Spiker, S., and Hall, T.C. (1994). The β-phaseolin gene is flanked by matrix-attachment regions. Plant J. **6,** 413–423.

Woodman, J.C., and Freeling, M. (1981). Identification of a genetic element that controls the organ specific expression of *Adh*1 in maize. Genetics **98,** 357–378.