Complex Structure of Knob DNA on Maize Chromosome *9*: Retrotransposon Invasion into Heterochromatin

E. V. Ananiev,* R. L. Phillips* and H. W. Rines*,†

**Department of Agronomy and Plant Genetics and Plant Molecular Genetics Institute, University of Minnesota, St. Paul, Minnesota 55108-6026, and †Plant Sciences Research Unit, U.S. Department of Agriculture–Agricultural Research Service, St. Paul, Minnesota 55108-6026*

Manuscript received October 27, 1997
Accepted for publication May 6, 1998

ABSTRACT

The recovery of maize (*Zea mays* L.) chromosome addition lines of *oat* (*Avena sativa* L.) from oat × maize crosses enables us to analyze the structure and composition of specific regions, such as knobs, of individual maize chromosomes. A DNA hybridization blot panel of eight individual maize chromosome addition lines revealed that 180-bp repeats found in knobs are present in each of these maize chromosomes, but the copy number varies from ~100 to 25,000. Cosmid clones with knob DNA segments were isolated from a genomic library of an oat-maize chromosome *9* addition line with the help of the 180-bp knob-associated repeated DNA sequence used as a probe. Cloned knob DNA segments revealed a complex organization in which blocks of tandemly arranged 180-bp repeating units are interrupted by insertions of other repeated DNA sequences, mostly represented by individual full size copies of retrotransposable elements. There is an obvious preference for the integration of retrotransposable elements into certain sites (hot spots) of the 180-bp repeat. Sequence microheterogeneity including point mutations and duplications was found in copies of 180-bp repeats. The 180-bp repeats within an array all had the same polarity. Restriction maps constructed for 23 cloned knob DNA fragments revealed the positions of polymorphic sites and sites of integration of insertion elements. Discovery of the interspersion of retrotransposable elements among blocks of tandem repeats in maize and some other organisms suggests that this pattern may be basic to heterochromatin organization for eukaryotes.

KNOBS are cytologically detectable heterochromatic components of pachytene maize chromosomes (McClintock 1929, 1959; Neuffer *et al.* 1997) which can be identified in somatic metaphase chromosomes with the help of differential staining techniques (Jewell and Islam-Faridi 1994). Their number, size, and position are variable in different maize varieties and are found in 23 possible locations on the 10 maize chromosomes (McClintock *et al.* 1981). Peacock *et al.* (1981) found that a 180-bp repeating unit arranged in tandem array is the major component of knob regions. Upon sequence analysis of about 20 copies of 180-bp repeats, a certain level of polymorphism was found among copies including a related 202-bp repeat that is a variant of the 180-bp repeat resulting from an internal 22-bp duplication (Dennis and Peacock 1984). A correlation between knob size and 180-bp repeat content was found by *in situ* hybridization. In addition, Viotti *et al.* (1985) speculated that there may be a number of other euchromatic sites with a low concentration of knob 180-bp repeats. Knob 180-bp repeat sequences were not found in centromeric heterochromatin or NOR heterochromatin. The proportion of 180-bp repeats in total genomic DNA was found to vary from 0.5% up to 6% in different maize varieties (Peacock *et al.* 1981; Rivin *et al.* 1986). The organization of 180-bp DNA repeats within individual knob sites is unknown except that they form clusters of tandem arrays and demonstrate a certain level of sequence heterogeneity (Dennis and Peacock 1984; Viotti *et al.* 1985).

Several genetic effects have been reported to be associated with knob size and numbers. Knob racial and geographic distributions are highly nonrandom. A positive correlation was found between the presence of knobs in certain chromosomes and later flowering time in maize (Chughtai and Steffensen 1987). Some, and possibly all, knobs affect the levels of recombination in particular regions of the chromosome complement, especially when heterozygous. For example, the heterochromatin of the large knob (K10) on abnormal chromosome *10* has the remarkable property of causing preferential recovery of knobbed chromosomes from knobbed/knobless heterozygotes and enhancing recombination (Rhoades 1978). In most strains of maize, the knobs are inactive and lag behind the true centromeres at anaphase. However, when abnormal *10* (K10) is present, knobs form "neocentromeres" that are pulled ahead of the true centromeres (Rhoades 1978). Loss of terminal chromosomal segments from knobbed A

Corresponding author: Ronald L. Phillips, Department of Agronomy and Plant Genetics, University of Minnesota, 411 Borlaug Hall, 1991 Upper Buford Circle, St. Paul, MN 55108-6026.
E-mail: phill005@maroon.tc.umn.edu

chromosomes in the second microspore division (Rhoades and Dempsey 1973) was observed in the presence of two or more B chromosomes. Genetic effects associated with knob DNA have been mostly attributed to only the 180-bp repeat. However, our data on the complex structure of knob DNA indicate that other types of DNA sequences like retrotransposons and other non-180-bp tandem repeats could be involved in control of the multiple functions associated with knob DNA.

Reconstruction of the physical structure of knob DNA in maize as well as any other heterochromatic regions composed of tandemly arrayed repeated sequences is difficult to achieve. This is because of the large size of heterochromatic blocks, their presence in different locations within a genome, and the presumably monotonous structure of the blocks, which are comprised of long tandem arrays of highly repetitive elements. The availability of oat-maize chromosome addition lines, each possessing an individual maize chromosome (Riera-Lizarazu *et al.* 1996), provides a unique opportunity to study knob DNA structure. A reduction in complexity of total maize genomic DNA occurs by having present only 1 out of 10 maize chromosomes. This allows the separation of one knob from the rest and has enabled us to study the structure and composition of a knob on maize chromosome *9*.

MATERIALS AND METHODS

DNA purification from maize and oat strains: Oat-maize addition lines for maize chromosomes *2–9* were produced from plants recovered following sexual crosses of oat by maize (Riera-Lizarazu *et al.* 1996). The presence and identity of the maize chromosomes in these lines had been determined by genomic *in situ* hybridization of root-tip cells and by Southern hybridization using a set of known mapped maize RFLPs. The presence of a maize chromosome was verified cytologically. DNA was purified from the chromosome addition lines, the maize lines "Seneca 60," "A188," "Gaspe," and "N28," and the oat lines "Starter-1" and "Sun II." Isolation and analysis of DNA were performed as described earlier (Ananiev *et al.* 1997).

Copy number estimation of the 180-bp repeat: For the Southern blot panel, genomic DNA of maize, oat, and the eight maize chromosome addition lines was cut with *Hae*III, which has one recognition site in the 180-bp knob repeat. DNA concentrations for all lines were adjusted according to genome equivalents. Maize genomic DNA was diluted sixfold in comparison with the oat genomic DNA, as the maize genome is 2400 Mb and the oat genome is ~12,000 Mb. Dilutions of DNA samples of a plasmid (cut with *Hin*dIII) with one copy of the 180-bp repeat (Peacock *et al.* 1981) and of a cosmid (cut with *Nde*I) with a 34-kb insertion composed entirely of 180-bp repeats were loaded on the same gel as standards for comparison. Hybridization was performed at standard conditions (Helentjaris 1995) at 65° overnight with consecutive washes in 2× SSC (0.3 m NaCl, 0.03 m Na acetate) and 0.1× SSC solutions at 65°. Densitometry of ethidium bromide gels and autoradiographs after different exposure times was performed with the help of Image software (National Institutes of Health, Bethesda, MD).

Cosmid library construction and screening: Cosmid library construction and screening were done according to protocols provided by the manufacturer (Stratagene, La Jolla, CA) of the cosmid vector and packaging extracts. Total nuclear DNA of oat-maize chromosome *9* addition line genomic DNA was partially digested with *Sau*3A, dephosphorylated, and ligated to the cosmid vector SuperCos1. The ligation products were packaged using GigaPack II packaging extract and the library was propagated in *Escherichia coli* XL1-Blue MR. The library was screened with the labeled 180-bp knob DNA sequence and clones which gave positive signals were isolated. Cosmid DNA with cloned knob DNA was cut with appropriate restriction enzymes and the presence of knob 180-bp DNA verified by Southern blot hybridization. Some of the DNA subfragments were recloned in pBS/KS or in SuperCos1 for subsequent sequencing or additional analysis. PCR amplification of different segments of cloned DNA was performed according to standard protocol (Sambrook *et al.* 1989).

DNA analysis: Gel-blot analysis of plant and cosmid DNA after fractionation in regular and CHEF pulsed-field electrophoresis systems was carried out as described by Sambrook *et al.* (1989) with several modifications (Helentjaris 1995). DNA fragments and total plant DNA were labeled by random primer extension (Feinberg and Vogelstein 1984). A clone containing the maize 180-bp knob repeat (Peacock *et al.* 1981) was kindly provided by J. Peacock (Commonwealth Scientific and Industrial Research Organization, Canberra, Australia).

PCR amplification and DNA sequencing: A collection of probes for a number of retrotransposons was generated by amplification of corresponding DNA segments from genomic DNA using primers designed on the basis of published DNA sequences: Prem1 (Turcich and Mascarenhas 1994), Prem2 (Turcich *et al.* 1996), Huck1 (SanMiguel *et al.* 1996), Grande (Vincent and Martinez-Izquierdo 1996), Zeon 1 (Hu *et al.* 1995), Cin 1 (Shepherd *et al.* 1984), and Milt 1 (SanMiguel *et al.* 1996). We also used as probes a number of other maize repeated DNA sequences identified in the construction of a multiprobe for isolating maize-specific clones from a genomic library made from an oat-maize chromosome *9* addition line (Ananiev *et al.* 1997). To amplify the junctions between knob 180-bp repeats and other DNA sequences, primers were prepared complementary to different segments of the 180-bp repeat: K1, ACA CAA CCC CCA TTT TGT T; K2, CAT TGA TCA TCG ACC AGA C; K3, CGT CTG GTC GAT GAA CAAT; K4, AAA AAT GGG GGT TGT GTG GC; and to the LTR (Long Terminal Repeats) of retrotransposon Grande (13,779 bp) (Vincent and Martinez-Izquierdo 1996) (GenBank accession no. X976040). The latter are referred to as Grande LTR-Forward, AAA GAC CTC ACG AAA GGC CCA AGG; and Grande LTR-Reverse, AAA TGG TTC ATG CCG ATT GCA CG. The same primers were used to amplify DNA for sequencing certain segments of DNA directly from cosmid DNA, from recloned subfragments, or from PCR-amplified DNA segments. Sequencing was performed with the help of the *Taq* DyeDeoxy terminator cycle sequencing system (Applied Biosystems, Inc., Foster City, CA).

Restriction mapping with oligonucleotide probes: Restriction map construction was done according to the protocol adapted to the SuperCos1 vector following the manufacturer's recommendations. Cosmid DNA was cut with *Not*I to excise the insert flanked by the T3 and T7 promoter regions followed by partial digestion with an appropriate restriction enzyme. The digestion products were run on an agarose gel in regular or pulsed field gel electrophoresis (PFGE) systems, transferred to nitrocellulose, and probed with labeled oligonucleotide probe T3 (GGC CGC AAT TAA CCC TCA CTA AAG G) or oligonucleotide probe T7 (GGC CGC GAT ATA CGA CTC ACT ATA GG).

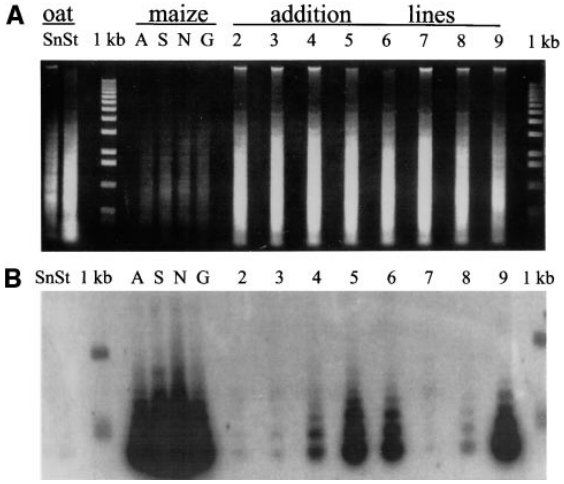

Figure 1.—Distribution and copy number estimation of 180-bp repeats among maize chromosomes. (A) Ethidium bromide-stained gel. DNA samples were cut with *Hae*III restriction enzyme. Oat lines Sun II (Sn) and Starter-1 (St); maize lines A188 (A), Seneca 60 (S), N28 (N), Gaspe (G); oat-maize chromosome addition lines 2–9; and 1 kb ladder. (B) Hybridization with 180-bp repeat as probe. Strong hybridization is seen with chromosomes *5*, *6*, and *9*; medium hybridization is seen with chromosomes *4* and *8*; and weak hybridization is seen with chromosomes *2*, *3*, and *7*.

RESULTS

Knob 180-bp repeat copy number in individual maize chromosomes: A hybridization blot panel of genomic DNA from eight oat-maize chromosome addition lines was prepared after complete digestion of the DNA with *Hae*III restriction enzyme (Figure 1A). This enzyme cuts tandem arrays of knob 180-bp repeats down to monomers. In addition, genomic DNA samples from two oat lines, Starter-1 and Sun II, and four maize lines, N28, Gaspe, Seneca 60, and A188, were treated the same way and loaded on the same blot panel for comparison. The blot panel was hybridized with the labeled 180-bp knob repeat. Analysis of the autoradiograms obtained after different exposure times revealed that all oat-maize chromosome addition lines gave positive hybridization with the 180-bp probe; no hybridization was detected with the oat parental lines. A large variation was observed in 180-bp repeat copy numbers in the different individual Seneca 60 maize chromosomes present as additions, ranging from about 100 copies each in chromosomes *2*, *3*, and *7*, up to 25,000 copies in chromosome *9* (Figure 1B, and Table 1). Different lines of maize contain different proportions of 180-bp repeats per haploid genome with about 35,000 copies in Gaspe and up to 150,000 copies in A188 and N28. Most of the hybridizing DNA fragments following complete digestion with *Hae*III are present in the form of monomer or dimer units of 180-bp repeats. However, a ladder-like hybridization pattern of 180-bp repeats up to ~5-mer units in all maize DNA samples indicates that there are point mutations in the *Hae*III recognition sites in some adjacent copies of 180-bp repeats.

TABLE 1

Copy number estimation of 180-bp knob repeats in oat, maize, and oat-maize chromosome addition lines

DNA sample	Copy no. of knob 180-bp repeats	Estimated size of knob DNA[a]	
		Based on copy no. of knob 180-bp repeats	Based on sum of *Eco*RI fragments
Oat lines			
Sun II	Not detectable	Not detectable	
Starter1	Not detectable	Not detectable	
Maize lines			
Seneca 60	50,000	10 Mb	
A188	150,000	30 Mb	
N28	150,000	30 Mb	
Gaspe	35,000	7 Mb	
Chromosome addition lines[b]			
AD2	≈100	≈20 kb	61 kb
AD3	≈100	≈20 kb	32 kb
AD4	1,000	200 kb	292 kb
AD5	11,500	2,300 kb	
AD6	2,500	500 kb	
AD7	≈100	≈20 kb	
AD8	500	100 kb	173 kb
AD9	25,000	5,000 kb	

[a] Size of the knob DNA in some of the maize chromosomes was estimated based on the copy number of knob 180-bp repeats and the sum of *Eco*RI fragments containing 180-bp repeats.

[b] AD refers to the chromosome addition line with the corresponding maize chromosome indicated by number.

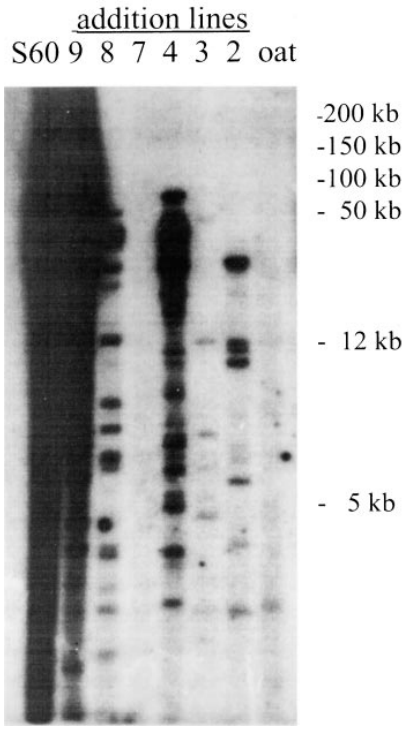

Figure 2.—CHEF pulsed field gel electrophoresis of DNA samples cut with *Eco*RI from oat-maize chromosome addition lines 2–4, and 7–9; and from parental maize (Seneca 60) and oat (Starter-1) lines. Multiple bands are seen in maize chromosomes *2–4*, *7*, and *8*. Chromosome *9* contains the largest knob and its large number of *Eco*RI fragments cannot be readily resolved as individual bands.

PFGE restriction fragment fingerprinting of knob DNA in maize chromosomes: DNA samples of several oat-maize chromosome addition lines, including ones for maize chromosomes *2–4*, *7*, *8*, and *9*, were cut with *Eco*RI and fractionated in a CHEF pulsed field electrophoresis system (Figure 2). Southern blot hybridization with the labeled knob 180-bp repeat as probe revealed a unique *Eco*RI pattern of DNA fragments for every maize chromosome. In Figure 2, which was obtained after two days of blot exposure, a few 180-bp positive *Eco*RI fragments are seen for chromosomes *2* and *3* while the large number of fragments in the lane with chromosome *9* makes counting the *Eco*RI fragments impossible. For chromosome *7*, a number of faint bands not visible in Figure 2 was observed but only after prolonged exposure time (10 days). DNA sequences of those 180-bp repeats present in GenBank Sequence Databases have no restriction sites for *Eco*RI restriction enzyme. However, the knob DNA in all maize chromosomes revealed a set of *Eco*RI fragments that varied in size from 1 kb to 100 kb. Summing the lengths of the *Eco*RI fragments, as could be done in some of the maize chromosomes, gave an independent estimation of the size of the chromosomal segments occupied by 180-bp repeats in each chromosome on the assumption that each band represents one DNA fragment (Table 1). The sum length of restriction fragments indicates that the overall size of the knob DNA regions may be up to three times larger than the size estimations based only on copy number of 180-bp repeats.

Complex structure of knob DNA from maize chromosome *9*: A cosmid library made from genomic DNA of an oat-maize chromosome *9* addition line was screened with a 180-bp knob repeat sequence. Hundreds of hybridization signals were detected on the library filter, which had about 300,000 clones. Twenty-three independent cosmid clones were isolated. The cloned segments of knob DNA varied in size from 30 to 48 kb. Cutting of these recombinant cosmids with a number of restriction enzymes revealed that the knob DNA has a more complex composition than might be expected if it were composed of only 180-bp repeats.

The restriction enzyme *Nde*I has one recognition site in the knob 180-bp repeat and no sites in the vector DNA. A simple restriction fragment profile consisting of one vector DNA fragment of ~6.7 kb and fragments of ~180 bp would be expected for recombinant cosmids composed entirely of 180-bp knob repeats. This profile was observed for only two clones, namely cosmids 1 and 3 (Figure 3). Twenty-one other clones had one or more additional DNA fragments of variable sizes. Southern blot hybridization of these recombinant clones with the labeled 180-bp repeat revealed strong hybridization to the 180-bp monomers, dimers or trimers, and very weak hybridization to the vector. Sequencing the ends of the vector-containing DNA fragments revealed that vector DNA is flanked by short segments of 180-bp repeating units extending out to the first *Nde*I restriction site. The additional fragments which vary in size from about 1 kb up to 14 kb in most cases are free from knob 180-bp repeats. However, some of them, such as the 14-kb fragments in cosmids 17–22 (Figure 3A), are flanked by short segments of 180-bp repeats up to the first *Nde*I restriction site (Figure 3, A and B). Hybridization of the same blot panel of cosmid clones with labeled total maize genomic DNA indicates that the additional fragments are moderately or highly repeated maize genomic DNA sequences (Figure 3C). The additional restriction fragments found in different cosmid clones were often similar or identical. For example, cosmids 17–22 all have the same large DNA fragment about 14-kb long. In total, 70% of the DNA in the recombinant clones are composed of 180-bp knob repeats while 30% of the DNA in these clones are other repeated DNA sequences.

Microheterogeneity of 180-bp repeats: Digestion of the cloned segments of knob DNA with a number of restriction enzymes, including some that have a recognition site within the 180-bp repeating unit, revealed a significant level of structural polymorphisms. Digestion of cloned knob DNA segments with *Nde*I and *Hae*III, both of which have one restriction site in the 180-bp repeat, revealed that monomers, dimers, and trimers of 180-bp repeats may be cut from the same recombinant

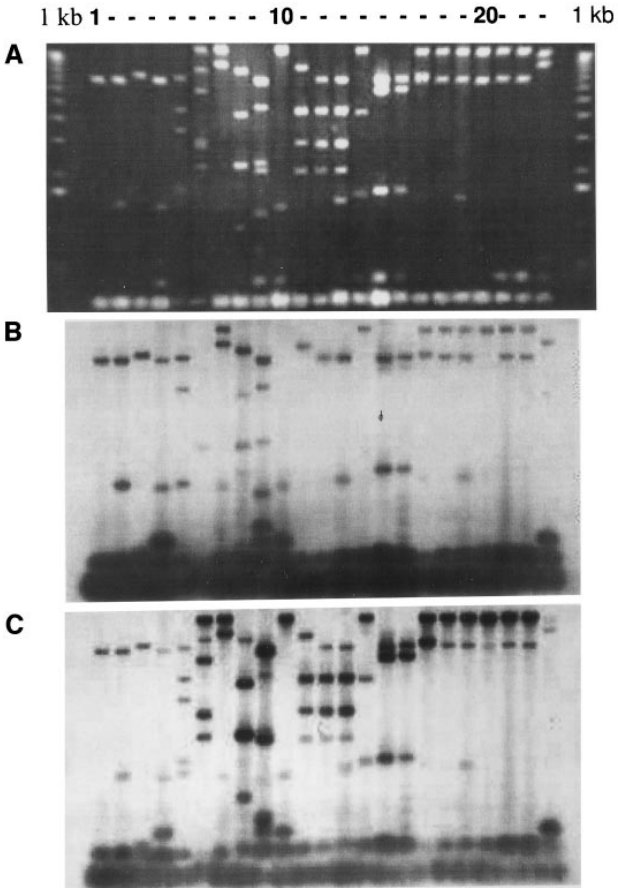

Figure 3.—Identification of insertion elements in cloned knob DNA segments. (A) Ethidium bromide-stained gel; cosmid clones (lanes 1–23) digested to completion with *Nde*I restriction enzymes, flanked by a 1-kb ladder. (B) Southern blot hybridization with 180-bp repeat as probe. Strong hybridization is seen with 180-bp monomers and oligomers; weak hybridization is seen with vector-containing DNA fragments (size of the vector is 6.5 kb) and some additional DNA fragments flanked by individual copies of 180-bp repeats; no hybridization is detected with a number of large *Nde*I subfragments (lanes 6, 10–14). (C) Hybridization with labeled maize genomic DNA as probe. Strong or moderate hybridization is seen for all *Nde*I DNA subfragments.

cosmid. Local variation appears to exist in the recognition sites in some of the adjacent copies of knob 180-bp repeats. Clones differ one from another according to their proportions of monomers, dimers, and trimers of 180-bp repeats (Figure 3). Some of them are composed mostly of monomeric units (cosmid 7) while others are enriched with dimers (cosmid 15) and trimers (cosmid 23).

Microheterogeneity of 180-bp repeats along different cloned segments was also seen in *Alu*I restriction analysis (data not shown). There were no *Alu*I restriction sites in previously reported 180-bp DNA sequences. Indeed in many cosmids there are *Alu*I DNA fragments up to 15–20 kb long that give a strong hybridization signal with the labeled 180-bp repeat. At the same time there are several recombinant cosmids with 180-bp knob DNA sequences that may be cut by *Alu*I into many fragments of variable sizes down to 100 bp. This observation indicates extensive nucleotide sequence polymorphism among copies of 180-bp repeat units within the same cloned segment of tandemly arranged 180-bp repeats. Comparison of the nucleotide sequences of 180-bp repeats showed a certain level of sequence polymorphism between adjacent 180-bp knob repeats (Figure 4) as well as between copies of 180-bp repeats isolated from different ends of the same cloned segment and from different recombinant cosmids. Variants of both 180-bp and related 202-bp repeats (Figure 4) were found in the same recombinant clone as well as in different recombinant clones, all of which originated from the large terminal knob of chromosome *9*.

Polarity of 180-bp repeats in tandem arrays: Both ends of five different DNA segments cloned in cosmids 1, 9, 15, 17, and 21 have been sequenced using primers complementary to the T3 or T7 promoter sequences adjacent to the cloning site in the SuperCos1 vector. In most cases up to three consecutive copies of 180-bp repeats may be identified in one sequencing reaction. The polarities of adjacent 180-bp repeats in ten sequenced DNA segments turned out to be the same as well as the polarity of the 180-bp repeats at both ends of the five cloned DNA fragments. In addition nine subfragments from different cosmid clones were cloned in the plasmid vector and their ends sequenced. In all cases the polarity of 180-bp knob repeats on opposite sides of cloned subfragments was found to be the same.

Arrangement of retrotransposable elements within the tandem arrays of 180-bp repeats: The presence of a recognition site for *Nde*I in almost every monomer unit of 180-bp repeat provides an opportunity to construct a high resolution restriction map that indicates the position and order of knob 180-bp repeats relative to other types of DNA sequences. Restriction maps for 23 cosmid clones were generated by a technique involving partial digestion of cloned DNA segments followed by blot-hybridization with labeled probes that are specific to the right and the left segments of the cloning site, respectively. The SuperCos1 vector (Stratagene) has two *Not*I restriction sites that flank the cloning site. *Not*I allows the release of an insertion with attached small fragments of vector corresponding to the T3 and T7 RNA polymerase promoters. Partial digestion of the *Not*I fragments with a second restriction enzyme, *Nde*I, will generate a ladder of restriction fragments, each band of which corresponds to a 180-bp monomeric unit in the regions of tandem arrays. On a stained gel all clones look alike because they form ladders of 180-bp repeats, which mask other DNA fragments (Figure 5A). However, blot hybridization with the labeled T3- (Figure 5B) or T7-oligonucleotides (Figure 5C) as probes enables the identification of a sequence of restriction sites in knob repeats from both ends of the insertion toward the center. A ladder-like pattern of hybridizing bands

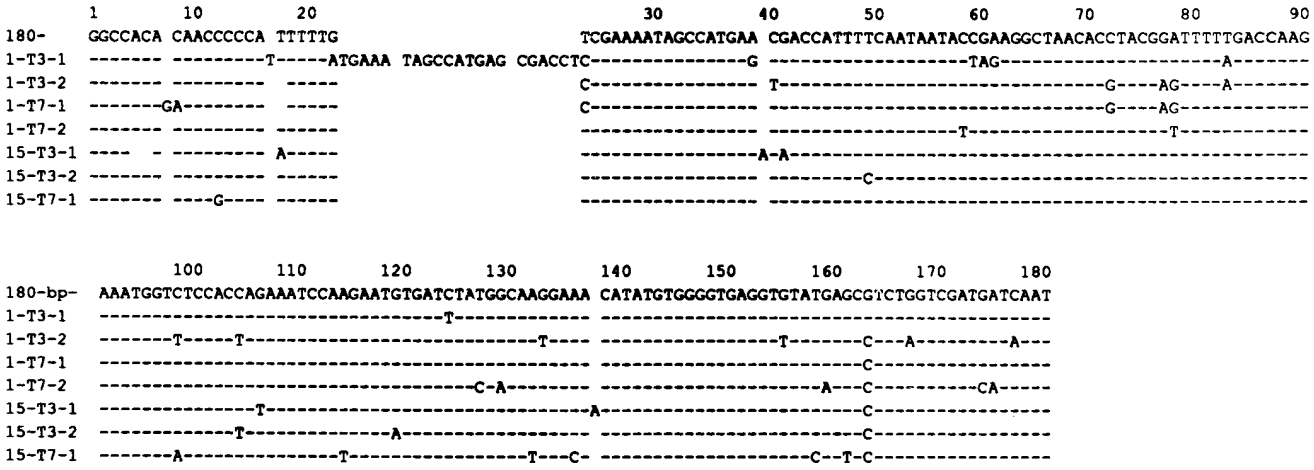

Figure 4.—DNA sequence comparison of the 180-bp repeat (Peacock *et al.* 1981) with seven 180-bp knob repeats from cosmid 1 and cosmid 15 (GenBank accession numbers are: AF030934, AF030935, AF030936, AF030937, AF030938, AF030939, and AF030940). The ends of the cloned knob DNA fragments were sequenced using the T3 and T7 primers that are complementary to the vector DNA around the cloning site. DNA sequences of one or two adjacent copies of 180-bp repeats were determined from each end. The first repeating unit (1-T3-1) at the T3 end of the cosmid 1 is actually a 202-bp variant of the 180-bp knob repeat resulting from the 21-bp duplication [92% identity to the 202-bp repeat described by Dennis and Peacock (1984)]. There is a certain level of polymorphism between adjacent copies of 180-bp repeats as well as between copies from different cloned DNA fragments that varies from 92 to 98% identity relative to the 180-bp repeat (Peacock *et al.* 1981).

indicates the regions with tandemly arranged 180-bp repeats while the long gaps correspond to the insertions of other types of DNA sequences. The ladder pattern itself demonstrates a certain level of microheterogeneity in the size of steps (sizes of repeating units) as well as the position of dimers and trimers within the ladder. For example, cloned knob DNA segments in cosmids 1 and 3 (Figure 5B) consist almost entirely of identical ladders of 180-bp repeats. However, an obvious shift in size of the step is seen in the 26th step of cosmid 1. Such minor changes in sizes of repeating units are obvious and can be used as markers for identification of specific regions in tandem arrays of 180-bp repeats. Clones 15 and 16 (Figure 5C) show variant positions of a large insertion element. For example, after the 7th step of the 180-bp repeat there are two long gaps with a combined size of about 7 kb in cosmid 15, which corresponds to one of the insertion elements. In cosmid 16, the same 7-kb gap is seen after about the 45th 180-bp repeat. Large insertion elements may be found in the middle or at the flanking regions of the cloned fragments. In some cloned DNA fragments (cosmids 7, 9, and 17) there are copies of two different insertion elements (Figure 6) that are separated by tracks of 180-bp repeats. In cosmid 6 a block of three different insertion elements was found flanked by knob 180-bp repeats.

A combination of regular gel electrophoresis and pulsed field gel electrophoresis (data not shown) was applied for fractionation of the resulting DNA fragments to enable restriction mapping from each end through the whole length of the cloned DNA fragment in a cosmid. However, the resolution of a restriction map constructed with the help of this technique is better at the ends than at the middle of a cloned DNA fragment. Restriction maps were constructed for all 23 cloned segments of knob DNA. These are schematically presented in Figure 6 to illustrate the relative position of the insertion elements within the arrays of 180-bp repeats. These 23 clones represent only 10–20% of the knob DNA from chromosome *9* (Table 1). Each of them has a specific distribution of insertion elements within the arrays of 180-bp repeats as well as a characteristic microheterogeneity of 180-bp repeats (Figure 5). Eventually this approach may allow the identification of overlapping segments in different recombinant clones and enable the reconstruction of a physical map for the entire knob region.

Abundance of retrotransposons embedded in the 180-bp knob tandem arrays: Some of the *Nde*I subfragments that were free from 180-bp knob repeats were isolated from recombinant cosmids and were used as labeled probes in hybridization experiments with the same blot panel of cosmid clones. At least seven different families of insertion elements were found in different cloned knob DNA segments (Table 2).

The insertion elements from cosmid clones with knob repeats of maize chromosome *9* were probed with the DNA sequences of known maize dispersed repetitive elements and retrotransposons (Table 2). The probes for these retrotransposable elements were generated with the help of PCR amplification of corresponding DNA segments using as a template total maize genomic DNA and pairs of primers which are complementary to the internal regions or to the LTRs of a specific retrotransposable element. Some of the insertion ele-

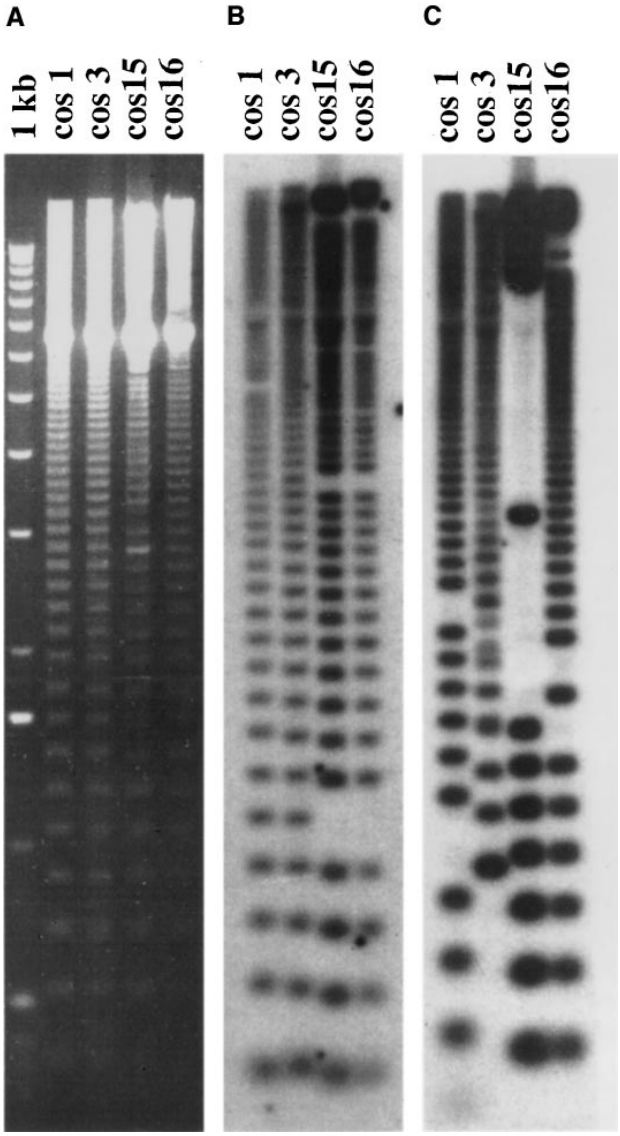

Figure 5.—Restriction mapping of cloned knob DNA segments. Recombinant cosmids with knob DNA segments were digested to completion with *Not*I and partially digested with *Nde*I and fractionated in a 0.7% agarose gel. (A) Ethidium bromide-stained gel shows a quite uniform ladder-like pattern of restriction fragments in all four samples of DNA from cosmids 1, 3, 15, and 16; (B) Southern blot hybridization with T3-oligo; (C) Southern blot hybridization with T7-oligo. Restriction maps for cosmids 1 and 3 look identical from the T3-end; however, at the 26th step of the 180-bp repeat ladder in cosmid 1 there is a gap corresponding to a dimer (B). Such a gap may indicate the presence of two adjacent 180-bp repeats which lack the *Nde*I recognition site that would normally separate them. Restriction maps for cosmids 1 and 3 look different from the T7-end (C). There is an obvious shift in the position of the *Nde*I sites relative to the cloning site in these two knob DNA sequences in cosmid 1 and cosmid 3. Presumably a step made of dimers of 180-bp repeats is seen after the 3rd and 10th steps in cosmid 1. A trimer of 180-bp repeats is seen at the beginning of the ladder in cosmid 3. Restriction maps for cosmids 15 and 16 look identical from the T3-end and different from the T7-end. The large gap ~7-kb long in cosmid 15 after the 7th step corresponds to the insertion element Zeon 1 with one internal *Nde*I site. The same gap produced by the insertion element Zeon 1 is seen in cosmid 16 after about the 45th step.

ments found in knob DNA were revealed to be copies of known retrotransposable elements, such as Zeon, Grande, and Prem2. At least two new insertion elements, which we named repeated element (RE)-15 (15 kb long) and RE-10 (10 kb long), were found that have no homology to the available set of maize repeats (Table 2). Several other DNA segments containing unknown maize repeated DNA sequences were found including relatively large (7–10 kb) segments in cosmids 9 and 23 and relatively small (1.0–1.5) segments in cosmids 2, 4, and 22 (Figure 6).

The insertion elements identified in cosmid clones with knob DNA were hybridized to the blot panel of chromosome addition lines and to an arranged partial cosmid library (1536 clones) of maize genomic DNA to determine the chromosome distribution of these elements and their copy numbers in the maize genome. For example, retroelement Grande gave a distinct banding pattern with every maize chromosome on the blot panel of maize-oat chromosome addition lines (Figure 7A) and highlighted about 11% of all cosmid clones in an arranged library of 1536 maize clones (Figure 7B). These data indicate that there are about 6000 copies of this element dispersed along the maize chromosomes.

Hybridization of RE-10 and RE-15 to the blot-panel of chromosome addition lines and to the arranged library of cosmid clones with maize genomic DNA revealed that these insertion elements are dispersed throughout all maize chromosomes and are medium copy number DNA sequences (5000–10,000 copies per haploid genome) (data not shown). RE-15 was partially sequenced and revealed no matches in the GenBank sequence databases.

The insertion elements found in the cloned knob DNA segments are close in size to full size copies of the corresponding retrotransposable elements. The retrotransposon Grande (13,779 bp long; Vincent and Martinez-Izquierdo 1996) was found in eight independently isolated cosmid clones. In five of them, the size of the element is about 14 kb. In three other recombinant clones only a portion of the element is present and is located at the flanks of the cloned DNA segments, presumably because it was cut with the restriction enzyme (*Sau*3A) in the process of cloning.

In the process of characterizing maize repeated DNA sequences (Ananiev *et al.* 1997), one family of repeats, RE-2, was identified that is probably the most abundant type of dispersed DNA sequences we found in the maize genome. It was found in 80% of the cosmid clones originating from chromosome 9 and in 60% of all clones in the cosmid library of maize genomic DNA. This element hybridizes to the same cosmid clones in our arranged maize genomic cosmid library (1536 clones) as do the LTRs of Huck retrotransposable elements (SanMiguel *et al.* 1996), indicating that these two probes represent the same retrotransposable element. However, no copies of this retrotransposon were found

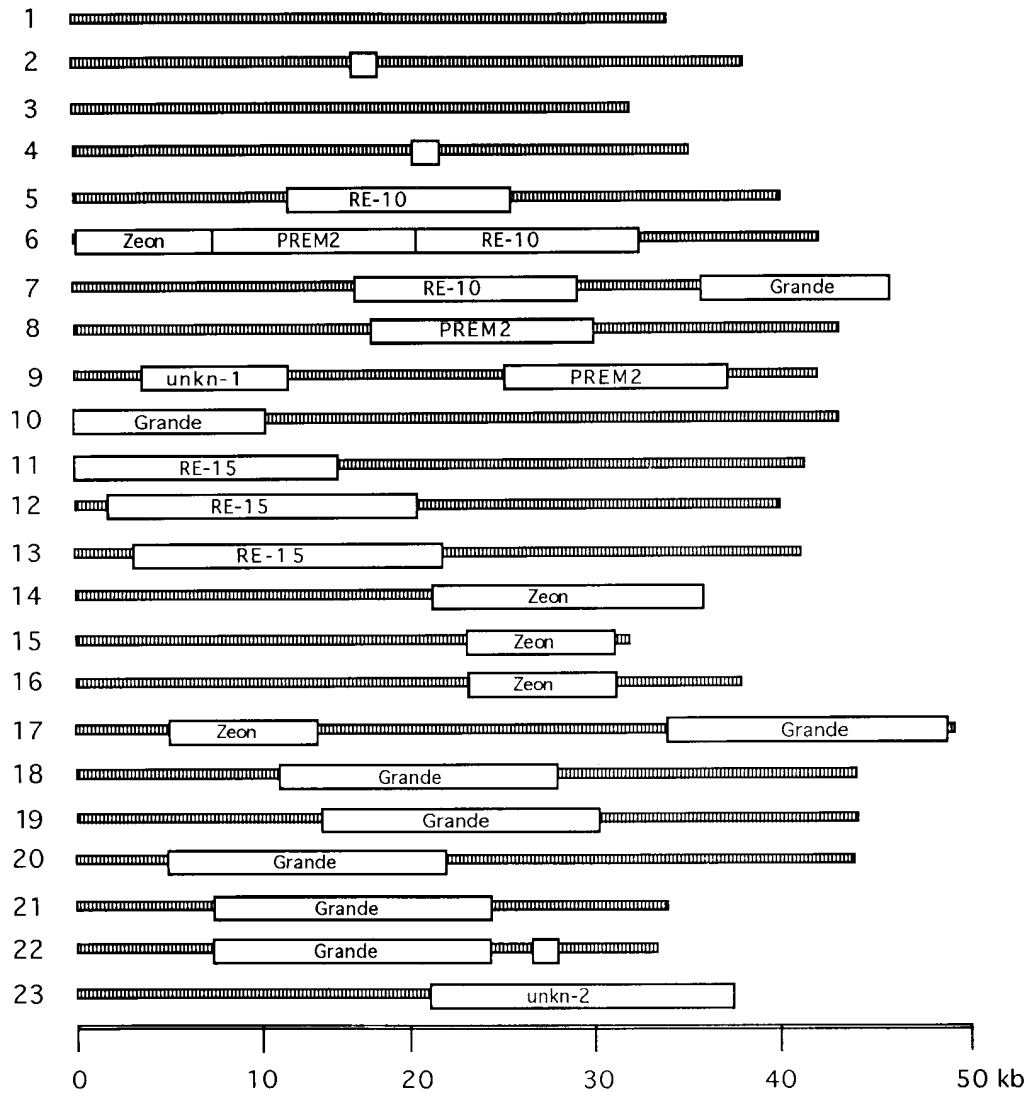

Figure 6.—Schematic representation of cloned knob DNA segments based on restriction maps constructed according to the protocol adapted to the SuperCos1 vector. Cosmid DNA was cut with *Not*I to completion and partially digested with *Nde*I. DNA samples were fractionated in agarose gels in standard and PFGE systems, blotted and hybridized with labeled oligos complementary to the T3 and T7 promoters. In addition, blot panels of cosmid clones with knob DNA cut to completion with *Nde*I were tested with a number of probes specific for different repeated elements of the maize genome. These experiments enabled identification of most of the insertion elements and determination of their position within the tandem arrays of 180-bp repeats. Hatch-marked regions indicate blocks of tandem 180-bp repeats; open regions indicate insertions of other elements (see Table 2).

within 23 cloned knob DNA segments. The frequent occurrence of Grande elements and failure to detect Huck elements in any of the 23 cosmids with knob DNA we isolated may indicate that certain retrotransposons have different patterns of distribution in the maize genome.

Site specificity of retrotransposable element integration within the 180-kb repeat: Several strategies were used to identify the sites of integration of insertion elements within the 180-bp repeat. Some of these elements may be cut out from the cloned knob DNA segment with the help of the *Nde*I restriction enzyme because they have no internal restriction sites for *Nde*I. Presumably the closest *Nde*I restriction sites are located in adjacent copies of 180-bp repeats. Recloning one of those elements into another vector and sequencing the ends allowed the identification of the integration site for a Grande element. Another approach is based on amplification of the integration site by PCR with the help of two primers, one of which is specific for the LTR of a transposable element and the other specific to the 180-bp repeat. For some copies of Grande elements, such a combination of primers was found and a corresponding integration site was amplified and then sequenced.

There appears to be a preference site (hot spot) of integration of retrotransposable elements in the 180-bp repeat. For example, three different insertion elements, Grande, Zeon, and RE-15 (Figure 8), were found each inserted at the same site in 180-bp repeat sequences. In five different cosmids, the copies of Grande element were found integrated in the same orientation and at position 8 of 180-bp repeat sequences (Figure 8A). In four cases, a 5-bp duplication of the integration site was found. Retroelement Zeon was found integrated practically at the same site at position 7 (Figure 8B). One end of the insertion element RE-15 (Figure 8C) was found also integrated at position 8 in the 180-bp repeat while the other end was at position 135 of the 180-bp repeat; 127 bp of the knob repeat were deleted.

In cosmid 9 the PREM2 element is integrated at position 100 and has a 5-bp duplication at the integration site (Figure 8D). Comparison of nucleotide sequences

TABLE 2

180-bp repeat and insertion element composition of knob repeat-containing cosmid clones from maize chromosome *9*

Cosmid	Size of cloned segment (kb)	180-bp block (kb)	Cross-hybridization to probes[a]	Size of insertion elements (kb)
1	32	32	—	—
2	36	36	—	—
3	30	30	—	—
4	38	38	—	—
5	38	29	RE-10	10
6	40	>10	Zeon	7
			PREM2	8
			RE-10	10
7	42	24	Grande	>9
			RE-10	10
8	41	30	PREM2	11
9	40	26	Unknown-1	7
			PREM2	>7
10	41	33.5	Grande	8.5
11	39	25	RE-15	14
12	38	23	RE-15	15
13	39	23	RE-15	15
14	33	20	Zeon	13.1
15	30	24	Zeon	7
16	36	30	Zeon	7
17	48	27	Zeon	7
			Grande	14
18	42	28	Grande	14
19	42	28	Grande	14
20	42	28	Grande	14
21	31	17	Grande	14
22	31	17	Grande	14
23	34	18	Unknown-2	15.5

[a] DNA fragments corresponding to different maize repeated DNA sequences were generated via PCR amplification with the help of primers based on published DNA sequences (see text and materials and methods). Sequences of Huck1, RE-2, and Milt were not found in this set of clones.

of the 180-bp repeats and retrotransposable elements at junction sites in different cosmid clones revealed a certain level of microheterogeneity, which indicates that these are independent integration events of different copies of retroelements (Figure 8, A–C).

DISCUSSION

Advantage of oat-maize chromosome addition lines for analysis of specialized chromosomal regions: Analysis of the structure and function of heterochromatic regions is difficult. The special problems relate to the large size and number of blocks of heterochromatin at different genomic locations, and presumably their monotonous structure of long tandem arrays of highly repetitive elements. It is difficult to dissect heterochromatin by classic genetic methods. Sequences of different satellite DNA repeats and their proportion in the genome have been well characterized, but the nature of their organization in heterochromatin is only roughly known (Lohe *et al.* 1993).

The oat-maize chromosome addition lines, which were generated earlier (Riera-Lizarazu *et al.* 1996), provide a unique opportunity to study the DNA of a single maize chromosome. Having one maize chromosome in an oat-maize addition line leads to a 10-fold reduction in complexity of the maize genome. Earlier we proposed using the oat-maize chromosome addition lines as a new system for the physical mapping of maize chromosomes (Ananiev *et al.* 1997). We found that maize and oat genomes are composed mostly of diverged families of repeated elements. In addition, we found that 80% of medium repetitive sequences and up to 50% of low copy and unique sequences are also species-specific (maize relative to oat). We developed a multiprobe as a composite of maize-specific dispersed highly repetitive DNA sequences. This multiprobe was used to isolate maize-specific clones from a cosmid library made from an oat-maize chromosome *9* addition

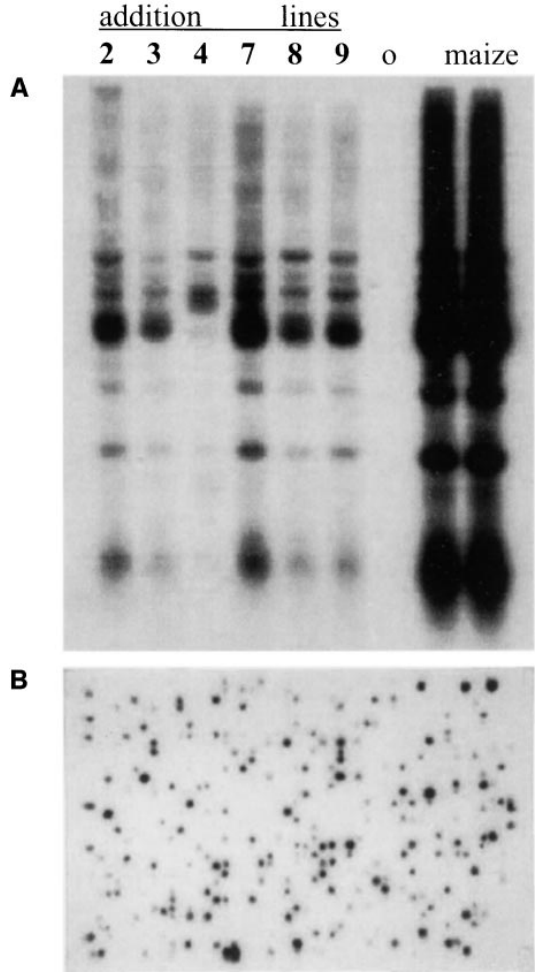

Figure 7.—Southern blot hybridization of the Grande 4-specific probe to a blot panel of (A) oat-maize chromosome addition lines and to (B) a partial arranged cosmid library (1536 clones) of maize genomic DNA (Seneca 60).

(A) **Grande**

```
Cos          180-bp-knob ------ 4-------8  13,774-----  Grande
17  - TCGATGATCAATGGCCACAC--TGTCGGTGTTTTGGGTCCGACCGCA -
18  - TCCATGATCAATGGCCACAC--TGTCGGTGTTTTGGGTCCGACCGCA -
19  - TCGATGATCAATGGCCACAC--TGTCGGTGTTTTGGGTCCGACCGCA -
20  - TCGATGATCAATGGCCACAC--TGTCGGTGTTTTGGGTCCGACCGCA -

                          Grande -1  4------8  -------180-bp-knob
17 - CCCAGGGGATATTTATCCCCCACA--CACACAACCCCCATTTTTGTC-
18 - CCCAGGGGATATTTATCCCCCACA--CACACAACCCCCATTTTTGTC-
19 - CCCAGGGGATATTTATCCCCCACA--CACACAACCCCCATTTTTGTC-
20 - CCCCGGGGATATTTATCCCCCACA--CACACAACCCCCATTTTTGTC-
 7 - CCCAGGGGATATTTATCCCCCACA--CACACAACCCCCATTTTTGTC-
```

(B) **Zeon**

```
                    180-bp ----- 4  ---7  1------  Zeon
15  -TCNATGATTAATGGCCACA--TGTTGGGGACTTGTTCTCAAGTGCTA-
15 -GACGCCGAAGGTCCCCAACA--CCACACAACCCCCATTTTTGTCG-
                  Zeon------7261     4--------8-----  180-bp knob
```

(C) **RE-15**

```
                180-bp-knob  4-------8     1-RE-15
12 -TCGATGATAAATGGCCACAC--agctgTGGCGGAACCACCCCAATTATT-
12 - CATCAGTCTGCGGGTGGTAagctg---AACATATGTGGGGTGAAGGTGT-
                                RE-15   135 --- 180-bp
```

(D) **PREM2**

```
                180-bp--knob --96---100    1----PREM2
9  -TTTTGACCAAGAAATGGTCTC--TGAAACGGAATTAGGCTCACACCTATT-
9  - ACCCCCCCTCTAGGCGACTTCCA--GTCTCCTCTAGAAATCCAAGAAT-
                  PREM2 -----9444     96---100----180-bp--knob
```

Figure 8.—Integration sites for different insertion elements (retrotransposons) in 180-bp repeats. (A) The integration sites for retrotransposon Grande 4 coincided in five different knob DNA segments. Upon integration this element generates a 5-bp duplication of the integration site at position 8 in the 180-bp repeat. (B) Retrotransposon Zeon 1 integrates at position 7 and generates a 5-bp duplication as well. (C) The element RE-15 is integrated at position 8 of the 180-bp repeat at one flank and at position 135 at another flank. Small letters indicate short 5-bp direct repeats that presumably belong to the RE-15 elements. (D) Upon integration the retrotransposon PREM2 generates a 5-bp duplication of the integration site at position 100 in the 180-bp repeat in cosmid 9. Numbers at the beginning of each nucleotide sequence indicate a corresponding cosmid. Bold font designates the 180-bp knob repeat sequence.

line (Ananiev *et al.* 1997). This approach allowed us to generate a maize chromosome *9*-specific library. It was also possible to use this system for the isolation of recombinant cosmid clones with DNA fragments from a particular region of a chromosome, such as knob DNA from maize chromosome *9*. The cosmid library used in this research contains about four maize chromosome *9* equivalents. Potentially it is possible to isolate a set of cosmid clones that will represent the entire knob DNA region. In this research we analyzed only 23 cosmid clones, which probably represent 800 kb of knob DNA or ~10–20% of the knob DNA on chromosome *9*.

Seneca 60 is a parent maize line used in crosses to produce the oat-maize chromosome addition lines. Cytologically, chromosome *9* in the maize variety Seneca 60 has only one prominent knob that is located on the short arm (Laurie and Bennett 1985) and presumably is the residence of 180-bp repeats. However, the possibility of small clusters of the 180-bp repeats or single copies dispersed along the maize chromosome cannot be excluded. We used our collection of 200 cosmid clones derived from chromosome *9* in a hybridization experiment to test for the presence of solo copies of the 180-bp repeats and found none. This observation indicates that 180-bp knob repeats most likely are not dispersed throughout chromosome *9*. However, our data do not completely exclude the possibility that another small knob site(s) may exist somewhere on chromosome *9*.

Complex structure of heterochromatin and diverse genetic effects related to knob DNA: A blot panel of eight individual maize chromosome addition lines revealed that knob 180-bp repeats are present in all maize chromosomes, but the copy number per chromosome varies from 100 to 25,000. Restriction fragment fingerprinting of knob DNA in different maize chromosomes by Southern blot hybridization with the 180-bp repeat as probe revealed a unique organization of knob DNA in each chromosome. We found that basic 180-bp knob repeats form tandem arrays of different lengths that are interrupted by different types of other repeated sequences. Direct sequencing of adjacent copies of 180-

bp repeat and high resolution restriction mapping of cloned knob DNA segments revealed a certain level of microheterogeneity, namely point mutations, small deletions, and insertions within the 180-bp units. The 180-bp repeats together with occasional repeats of a 202-bp sequence—a variant of the 180-bp repeat resulting from an internal 22-bp duplication (Dennis and Peacock 1984)—were found in the recombinant clones that originated presumably from the single knob of chromosome *9*.

At least five different dispersed repeated sequences were found in association with knob 180-bp repeats. These five are found on all maize chromosomes studied and are dispersed throughout euchromatic regions.

Our data on the complex structure of knob DNA in maize chromosome *9* in combination with restriction fingerprinting of knob DNA on a blot panel of chromosome addition lines suggest that knobs on other maize chromosomes also may have a complex organization. Analysis of knob DNA from different maize chromosomes will allow a direct comparison of DNA organization in different knobs as well as the characterization of chromosomal DNA sequences that flank knobs. The complex organization of knob regions suggests that besides 180-bp repeats other types of genetic elements also may be involved in the control of specific functions associated with knob DNA.

Retrotransposon invasion in blocks of tandem repeats as a basic pattern of heterochromatin organization: The accumulation of retrotransposable elements in heterochromatin was first demonstrated by Ananiev *et al.* (1978, 1984) in *Drosophila melanogaster*. Comparative analysis of the distribution of 12 different families of dispersed repetitive elements in salivary gland chromosomes revealed preferential accumulation of retrotransposable elements in centromeric heterochromatin and in intercalary heterochromatin. Molecular cloning and restriction mapping of large centromeric segments indicated that Drosophila heterochromatin is composed of alternating blocks of simple satellite DNA and complex DNA or copies of individual retrotransposons (Le *et al.* 1995). Zhang and Spradling (1995) found that middle repetitive and unique sequence DNAs are interspersed with satellite DNAs in mitotic heterochromatin. They found that the active transposable *P* element shows a 2-fold preferential integration into heterochromatin of the *Y* chromosome (Zhang and Spradling 1994). The centromeric regions of human chromosomes may contain complex DNA, such as Alu and LINE elements (Wevrick *et al.* 1992). Preferential integration of retroviral elements in constitutive, late replicating heterochromatin was found in the Hamster genome (Taruscio and Manuelidis 1991). Yeast retrotransposable element Ty5 was found preferentially integrated into regions of silent chromatin at telomeres and mating type loci (Zou *et al.* 1996). Recently, Pelissier *et al.* (1996) found that the retroelement Athila frequently associated with a 180-bp repeat in Arabidopsis. The Arabidopsis 180-bp repeat is unrelated to the maize 180-bp repeat but is tandemly arrayed in pericentromeric regions of all chromosomes.

In this research we found that knob DNA, which was considered as long monotonous stretches of tandem repeats (Peacock *et al.* 1981), contains interspersed members of at least three known families of retrotransposable elements identified in the maize genome, namely: Grande, Zeon 1, and PREM2, and two other families of large insertion elements, RE-15 and RE-10. Thus, the retrotransposable elements are the major factor that disrupts this homogeneous organization. According to our data the retrotransposable elements comprise about 30% of cloned knob DNA fragments. The proportion of retrotransposable elements in euchromatic portions of the maize genome (or in a random set of cosmid clones) is at least twice as high as in knobs. Thus, in general, retrotransposable elements are underrepresented in knob regions in comparison with their proportion in the entire maize genome. However, the retrotransposon Grande was found three times more often in the knob DNA than in a random set of cosmid clones while the retrotransposon Huck, the most abundant repeated element in the maize genome, was not found in any of 23 cloned knob DNA fragments. This observation allowed us to speculate that the integration of a retroelement into knob DNA may be considered as an indication of a "recent" event of retrotransposition on an evolutionary scale. From this point of view the Grande element may be considered as an active retroelement and Huck as an inactive one. However, we cannot exclude that the retrotransposon Huck may have different target specificity. Similarity of heterochromatin structure in distantly related eukaryotic organisms like maize, Drosophila and human implies that interspersion of tandemly arranged short repeated sequences with retrotransposable elements is a fundamental pattern of heterochromatin organization.

Preferential integration of retrotransposable elements into particular sites of the knob 180-bp repeat: Invasion of retrotransposable elements in heterochromatin as a physical process may be considered a property of retrotransposons; *i.e.*, the ability to integrate into any type of nucleotide sequence. However, preferential integration of retrotransposons into a specific site of the 180-knob repeat makes this particular heterochromatic sequence, the 180-bp repeat, a partner in this process and probably has its own rules. We found that the sites of integration of different insertion elements like RE-15, Zeon 1, and Grande into 180-bp repeats were nearly identical. At the same time the duplication of integration sites in euchromatic regions for Grande (AAGAG) (GenBank accession no. X97604) (Vincent and Martinez-Izquierdo 1996) is different from the duplication of the integration site (CACAC) in the 180-bp repeat. The PREM2 retroelement was found inserted into an-

other site of the 180-bp repeat and the duplication of integration sites in euchromatic regions for PREM2 (ATTAT) (GenBank accession no. U41000) (Turcich *et al.* 1996) also is different from the integration site in the 180-bp knob repeat.

What makes these particular sites so special for invasion of different retrotransposons? One can hypothesize that the knob 180-bp repeat has a specific physical structure at this particular site that allows it to serve as a receptor of extrachromosomal copies of retrotransposable elements. Integration specificity of retrotransposons has been found in many different species, like yeast, Dyctiostelium and mammals (Sandmeyer *et al.* 1990). In some cases the preferential integration sites are located in actively transcribed chromosomal regions and promoters; in others the preferential sites are located in repeated heterochromatic DNA sequences. The preferences may reflect nucleosome-free DNA segments, which might be more accessible for topoisomerases, nuclear matrix proteins, transcription factors or replication proteins (Sandmeyer *et al.* 1990). However, Pryciak *et al.* (1992) did not find preferential integration of murine leukemia virus in nucleosome-free or nuclease-sensitive regions. They proposed a model in which the integration machinery has preferential access to the exposed face of the nucleosomal DNA helix.

Accumulation of full-size copies of retrotransposons in knob DNA: The sizes of insertion elements that are found in knob DNA and that correspond to known retrotransposable elements are close to full-size copies of the corresponding elements. For example, Grande (13,779 bp long) (Vincent and Martinez-Izquierdo 1996) was found in eight independently isolated knob DNA-containing cosmid clones. In five of them, the element was about 14 kb and surrounded by 180-bp repeats. In three other recombinant clones, only a portion of the element was present probably because it was cut with the restriction enzyme (*Sau*3A) in the process of cloning. Similarly, Zeon (7,313 bp) and PREM2 (9,449) have homology to insertions of ~7-kb and 10-kb long, respectively, in a number of cosmid clones (Table 2). Sequence data indicate almost perfect homology between knob-associated and euchromatin-associated copies of Grande (Vincent and Martinez-Izquierdo 1996), as well as between knob-associated and euchromatin-associated copies for Zeon (Hu *et al.* 1995) and PREM2 (Turcich *et al.* 1996).

The accumulation of full-size nonrearranged copies of retrotransposons in knob regions is in sharp contrast with the organization of these elements within euchromatic regions where they are frequently truncated as the result of insertions of some other retrotransposons (SanMiguel *et al.* 1996). This fact may reflect recent (on an evolutionary scale) transposition events. Retrotransposable elements that are not found in knob DNA, like the Huck-retroelement, may be considered as inactive retrotransposons in the maize genome. We cannot exclude an option that knob DNA may serve as a repository for full-size copies of retroelements that may be protected by knob repeats and thus inaccessible for secondary integration of other transposable elements.

Conclusion: New information obtained in this research on the organization of knob-heterochromatin in maize raises several questions. These questions could be addressed by reconstruction of the physical structure of entire knob regions in several different chromosomes and in different maize lines in which knobs accounted for specific genetic effects. For testing any proposed hypothesis on possible mechanisms of knob variability, it is important to identify the actual physical structure of at least some knobs and the adjacent flanking chromosomal DNA regions and interspersed elements. Comparative analysis of knob organization in different chromosomes may allow identification of essential common features of knob structure.

The authors gratefully acknowledge the technical support of Ling Tong and Robert Brock. Funding support for the research was provided by U.S. Department of Agriculture (USDA)-National Research Initiative Competitive Grants Program grant no. 96-0087 and the Plant Molecular Genetics Institute, University of Minnesota. The paper is a joint contribution of the Minnesota Agricultural Experimental Station and USDA-Agricultural Research Service (ARS) published as Scientific Journal Series paper no. 971130028 of the Minnesota Agricultural Experimental Station. Mention of a trademark or proprietary product does not constitute a guarantee or warranty by USDA-ARS or the University of Minnesota and does not imply approval over other products that may also be suitable.

LITERATURE CITED

Ananiev, E. V., V. A. Gvozdev, Y. V. Ilyin, N. A. Tchurikov and G. P. Georgiev, 1978 Reiterated genes with varying location in intercalary heterochromatin regions of *Drosophila melanogaster* polytene chromosomes. Chromosoma **70:** 1–17.

Ananiev, E. V., V. E. Barsky, Y. V. Ilyin and M. V. Ryzic, 1984 The arrangement of transposable elements in the polytene chromosomes of *Drosophila melanogaster*. Chromosoma **90:** 366–377.

Ananiev, E. V., O. Riera-Lizarazu, H. W. Rines and R. L. Phillips, 1997 Oat-maize chromosome addition lines: a new system for mapping the maize genome. Proc. Natl. Acad. Sci. USA **99:** 3524–3529.

Chughtai, S. R., and D. M. Steffensen, 1987 Heterochromatic knob composition of commercial inbred lines of maize. Maydica **32:** 171–187.

Dennis, E. S., and W. J. Peacock, 1984 Knob heterochromatin homology in maize and its relatives. J. Mol. Evol. **20:** 341–350.

Feinberg, A. P., and B. Vogelstein, 1984 A technique for labeling restriction endonuclease fragments to high specific activity: Addendum. Anal. Biochem. **137:** 266–267.

Helentjaris, T., 1995 Neutral agarose gel electrophoresis, p. 9 in *UMC Maize RFLP Procedures Manual*, edited by UMC Maize RFLP Laboratory. University of Missouri, Columbia, MO.

Hu, W., O. P. Das and J. Messing, 1995 Zeon-1, a member of a new maize retrotransposon family. Mol. Gen. Genet. **248:** 471–480.

Jewell, D. C., and N. Islam-Faridi, 1994 A technique for preparation of somatic chromosome preparation and C-banding in maize, pp. 484–493 in *The Maize Handbook*, edited by M. Freeling and V. Walbot. Springer-Verlag, NY.

Laurie, D. A., and M. D. Bennett, 1985 Nuclear DNA content in the genera *Zea* and *Sorghum:* intergeneric, interspecific and intraspecific variation. Heredity **55:** 307–313.

Le, M., H. D. Duricka and G. H. Karpen, 1995 Islands of complex

DNA are widespread in *Drosophila* centric heterochromatin. Genetics **141:** 283–303.

Lohe, A. R., A. J. Hilliker and P. A. Roberts, 1993 Mapping simple repeated DNA sequences in heterochromatin of *Drosophila melanogaster*. Genetics **134:** 1149–1174.

McClintock, B., 1929 Chromosome morphology in *Zea mays*. Science **69:** 629.

McClintock, B., 1959 Genetic and cytological studies of maize. Carnegie Inst. Wash. Year Book **58:** 452–467.

McClintock, B., Y. Kato and A. Bluemenshein, 1981 *Chromosome Constitution of Races of Maize*. Colegio de Postgraduados, Chapingo, Mexico.

Neuffer, M. G., E. H. Coe and S. R. Wessler, 1997 Cytological map, p. 32 in *Mutants of Maize*. Cold Spring Harbor Laboratory Press, Plainview, NY.

Peacock, W. J., E. S. Dennis, M. M., Rhoades and A. J. Pryor, 1981 Highly repeated DNA sequence limited to knob heterochromatin in maize. Proc. Natl. Acad. Sci. USA **78:** 4490–4494.

Pelissier, T., S. Tutois, S. Tourmente, J. M. Deragon and G. Picard, 1996 DNA regions flanking the major *Arabidopsis thaliana* satellite are principally enriched in Athila retroelement sequences. Genetica **97:** 141–151.

Pryciak, P. M., A. Sil and H. E. Varmus, 1992 Retroviral integration into minichromosomes in vitro. EMBO J. **11:** 291–303.

Rhoades, M. M., 1978 Genetic effects of heterochromatin in maize, pp. 641–672 in *Maize Breeding and Genetics*, edited by B. D. Walden. Wiley and Sons, New York.

Rhoades, M. M., and E. Dempsey, 1973 Chromatin elimination induced by the B chromosome of maize. I. Mechanism of loss and the pattern of endosperm variegation. J. Hered. **64:** 13–18.

Riera-Lizarazu, O., H. W. Rines and R. L. Phillips, 1996 Cytological and molecular characterization of oat × maize partial hybrids. Theor. Appl. Genet. **93:** 123–135.

Rivin, C. J., C. A. Cullis and V. Walbot, 1986 Evaluating quantitative variation in the genome of *Zea mays*. Genetics **113:** 1009–1019.

Sambrook, J., E. F. Fritsch and T. Maniatis, 1989 *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Sandmeyer, S. B., L. J. Hansen and D. L. Chalker, 1990 Integration specificity of retrotransposons and retroviruses. Annu. Rev. Genet. **24:** 491–518.

SanMiguel, P. J., A. Tikhonov, Y.-K. Jin, N. Motchulskaia, D. Zakharov *et al.*, 1996 Nested retrotransposons in the intergenic regions of the maize genome. Science **274:** 765–768.

Shepherd, N. S., Z. Schwarz-Sommer, J. Blumberg vel Spalve, M. Gupta, U. Wienand *et al.*, 1984 Similarity of the Cin1 repetitive family of *Zea mays* to eukaryotic transposable elements. Nature **307:** 185–187.

Taruscio, D., and L. Manuelidis, 1991 Integration site preferences of endogeneous retroviruses. Chromosoma **101:** 141–156.

Turcich, M. P., and J. P. Mascarenhas, 1994 PREM-1, a putative maize retroelement has LTR (long terminal repeat) sequences that are preferentially transcribed in pollen. Sex. Plant Reprod. **7:** 2–11.

Turcich, M. P., A. Bokhari-Riza, D. A. Hamilton, C. He, W. Messier *et al.*, 1996 PREM-2 a copia-type retroelement in maize is expressed preferentially in early microspores. Sex. Plant Reprod. **9:** 65–74.

Vincent, C. M., and J. A. Martinez-Izquierdo, 1996 Has the *Zea* retrotransposon Grande 1 captured a cellular gene? (GenBank Accession No. X976040.)

Viotti, A., E. Privitera, E. Sala and N. Pogna, 1985 Distribution and clustering of two highly repeated sequences in the A and B chromosomes of maize. Theor. Appl. Genet. **70:** 234–239.

Wevrick, R., V. P. Willand and H. F. Willand, 1992 Structure of DNA near long tandem arrays of alfa satellite DNA at the centromere of human chromosome 7. Genomics **14:** 912–923.

Zhang, P., and A. C. Spradling, 1994 Insertional mutagenesis of *Drosophila* heterochromatin with single P elements. Proc. Natl. Acad. Sci. USA **91:** 3539–3543.

Zhang, P., and A. C. Spradling, 1995 The *Drosophila* salivary gland chromocenter contains highly polytenized subdomains of mitotic heterochromatin. Genetics **139:** 659–670.

Zou, S., N. Ke, J. M. Kim and D. F. Voytas, 1996 The Saccharomyces retrotransposon Ty5 integrates preferentially into regions of silent chromatin at the telomeres and mating loci. Genes Dev. **10:** 634–645.

Communicating editor: W. F. Sheridan