Proc. Natl. Acad. Sci. USA
Vol. 95, pp. 10785–10790, September 1998
Genetics

A knob-associated tandem repeat in maize capable of forming fold-back DNA segments: Are chromosome knobs megatransposons?

E. V. ANANIEV*, R. L. PHILLIPS*†, AND H. W. RINES*‡

*Department of Agronomy and Plant Genetics and Plant Molecular Genetics Institute, University of Minnesota, 1991 Upper Buford Circle, St. Paul, MN 55108; and ‡Plant Sciences Research Unit, U.S. Department of Agriculture, Agricultural Research Service, St. Paul, MN 55108

Contributed by Ronald L. Phillips, June 30, 1998

ABSTRACT **A class of tandemly repeated DNA sequences (TR-1) of 350-bp unit length was isolated from the knob DNA of chromosome 9 of *Zea mays* L. Comparative fluorescence *in situ* hybridization revealed that TR-1 elements are also present in cytologically detectable knobs on other maize chromosomes in different proportions relative to the previously described 180-bp repeats. At least one knob on chromosome 4 is composed predominantly of the TR-1 repeat. In addition, several small clusters of the TR-1 and 180-bp repeats have been found in different chromosomes, some not located in obvious knob heterochromatin. Variation in restriction fragment fingerprints and copy number of the TR-1 elements was found among maize lines and among maize chromosomes. TR-1 tandem arrays up to 70 kilobases in length can be interspersed with stretches of 180-bp tandem repeat arrays. DNA sequence analysis and restriction mapping of one particular stretch of tandemly arranged TR-1 units indicate that these elements may be organized in the form of fold-back DNA segments. The TR-1 repeat shares two short segments of homology with the 180-bp repeat. The longest of these segments (31 bp; 64% identity) corresponds to the conserved region among 180-bp repeats. The polymorphism and complex structure of knob DNA suggest that, similar to the fold-back DNA-containing giant transposons in *Drosophila*, maize knob DNA may have some properties of transposable elements.**

Knobs are cytologically observable heterochromatic regions present on maize chromosomes in locations that are characteristic of different lines. They have been the subject of investigations for decades since their discovery by B. McClintock (1). Knobs have been instrumental in providing proof for physical exchange of chromosomal segments in crossing over (2). They have provided data for phylogenetic studies of maize and related species (3–5). Knob numbers have been correlated with qualitative and quantitative traits in maize (6–11), and there is a correlation of knob numbers and DNA quantity per nucleus in different varieties of maize (12, 13). Knob heterochromatin has effects on the frequency of recombination (14) and on chromosome behavior in microspore divisions (15).

Peacock *et al.* (16) showed that a 180-bp repeating unit, arranged in tandem arrays, is a major component of knob regions. A correlation between knob size and 180-bp repeat content was found by *in situ* hybridization (17). Viotti *et al.* (18) speculated that, in addition, there may be euchromatic sites with a low number of 180-bp repeats. Knob 180-bp repeat sequences were not found in centromeric heterochromatin or in nucleolus organizer region heterochromatin. The 180-bp repeat was the only known family of repeated DNA sequences associated with knob heterochromatin. However, Ananiev *et al.* (19) recently isolated knob DNA sequences from a maize chromosome 9 alien addition line of oat (*Avena sativa* L.) and reported that maize knob DNA has a more complex organization. Stretches of tandemly repeated 180-bp knob DNA sequences were found to be interrupted by insertions of retrotransposable elements. These elements accounted for up to one-third of all DNA sequences in cloned knob DNA segments from chromosome 9. Further characterization of these chromosome 9 knob DNA segments revealed a stretch of DNA composed of a tandem array of a repeated DNA sequence ≈350 bp long. These tandemly arranged DNA repeats were termed TR-1 elements.

Comparative *in situ* hybridization to maize pachytene chromosomes using TR-1 elements and 180-bp repeats labeled with different fluorescent dyes indicated that these two classes of tandemly repeated DNA sequences are present in cytologically detectable knobs in different proportions relative to one another. Some knobs are composed mainly of TR-1 elements. In addition, several small clusters of TR-1 were found in other locations along the chromosomes. Stretches of TR-1 and 180-bp repeats were found to be interspersed. Sequence analysis revealed that tandem arrays of the TR-1 elements are capable of forming so called fold-back DNA segments or inverted DNA segments.

The most striking phenomenon associated with knobs is their high level of polymorphism in size, number, and distribution along the chromosomes of different strains of maize (5, 20–24). The origin of this polymorphism is still a mystery. We propose a hypothesis that maize knob DNA may be considered as megatransposons. This hypothesis takes into account the polymorphism and complex structure of knob DNA that arises from the presence of different types of retrotransposable elements and at least one family of fold-back elements composed of the newly identified TR-1 elements. Knob polymorphisms may have arisen from molecular rearrangements and transpositions of entire knob regions or DNA sequences within knob regions.

MATERIALS AND METHODS

Maize and Oat Strains. Plant materials used in this study included maize hybrid Seneca 60, maize inbred A188, oat cultivar Starter-1, and oat-maize chromosome addition lines for maize chromosomes 2, 3, 4, 7, 8, and 9. Oat–maize addition lines were derived from plants recovered after sexual crosses of oat by maize (25).

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviation: kb, kilobase.

Data deposition: The sequences reported in this paper have been deposited in the GenBank database (accession nos. AF071121 through AF071127).

†To whom reprint requests should be addressed.

DNA Purification and Analysis. Genomic DNA was purified as described (26). Cosmids containing maize knob DNA sequences were isolated from a cosmid library prepared from a maize chromosome 9 addition line in oat (19) that had been screened by using a labeled 180-bp knob repeat as a probe. A clone containing the maize 180-bp knob repeat (16) was kindly provided by J. Peacock (Commonwealth Scientific and Industrial Research Organization, Canberra, Australia). Gel-blot analysis of total genomic and cosmid DNA after regular and clamped homogeneous electric field (CHEF)-pulsed field gel electrophoresis was carried out as described by Sambrook *et al.* (27). Some of the fragments were subcloned into the plasmid vector pBluescript IIKS (Stratagene) for subsequent sequencing or additional analysis. Sequencing was performed with the help of the *Taq* DyeDeoxy terminator cycle sequencing system (Applied Biosystems).

Restriction Mapping with Oligonucleotide Probes. Restriction map construction was done according to the protocol adapted to the SuperCos1 vector following the manufacturer's (Stratagene) recommendations. Cosmid DNA was cut with *Not*I to excise the insert followed by partial digestion with an appropriate restriction enzyme. The digestion products were separated electrophoretically on an agarose gel in regular or pulse field electrophoresis systems, were transferred to nitrocellulose, and were 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).

***In Situ* Hybridization.** DNA samples were labeled, and *in situ* hybridization was performed according to the protocol provided by the manufacturer of the Prime-It Fluor Fluorescence Labeling Kit (Stratagene). DNA samples were labeled with fluorescein-12-dUTP or with tetramethylrhodamine-6-dUTP (Boehringer Mannheim). Chromosomes were counterstained in antifade solution [triethylenediamine (25 mg/ml) in a 1:1 (vol/vol) glycerol and PBS buffer] containing 20 ng/ml of 4′,6-diamino-2-phenylindole (DAPI) or 200 ng/ml of propidium iodide.

Pachytene chromosomes were prepared from microsporocytes of the maize line Seneca 60. Anthers were fixed in 3:1 (100% ethanol: glacial acetic acid) overnight and were stored in 70% ethanol. To prepare spreads of pachytene chromosomes, anthers were transferred into 45% acetic acid for 5 min. Microsporocytes at the appropriate stage of meiotic prophase were squashed in 45% acetic acid, the slides were frozen in liquid nitrogen, the cover glasses were popped off with a razor blade, and the slide was fixed in absolute ethanol overnight and was dried and stored until used for *in situ* hybridization.

RESULTS

Isolation of a 350-bp Knob-Associated Repeat. In the process of characterizing 23 cosmid clones containing tandem arrays of 180-bp repeats from the knob of maize chromosome 9 (19), one clone was found to contain a 6.5-kilobase (kb) *Hae*III fragment composed of different repeated elements. *Eco*RI digestion of this fragment produced a group of fragments ≈0.35 kb each and two additional fragments ≈0.7 and 0.6 kb in length. Complete sequence analysis of four randomly chosen subclones of the smaller fragments revealed a high level of homology among the *Eco*RI fragments. These fragments represent a family of repeated DNA sequences (TR-1) with median size of 350 bp (Fig. 1). Three copies of these elements, 353, 357, and 358 bp in length (GenBank accession nos. AF071122, AF071123, and AF071124), have minor sequence differences whereas the fourth, 340 bp in length (accession no. AF071121), has a deletion of 20 bp. The 0.7-kb *Eco*RI subfragment (accession number AF071126) proved to be a dimer composed of two TR-1 repeats, 359 and 364 bp long, which had lost the *Eco*RI recognition site between them,

```
GAATTCAAAA CAATATATCA CCTTTTAAAC CTCAAATGTC   40
GTTTCTATGA TATTGAGGGC ATTGTATTCA CACGAGTGGG   80
TTTTAGAGTA CAACTAGTGG ATGAAGACAT TATTGGGCTA  120
GCTAACGATC TTTTGCGATA CCAGGTTTCC TATAATCCCC  160
TCTATTCTAG GCACACCAAG ATGGCATTTC TGTCCACTCA  200
AGTAAAACAC CACACATCAA TATGCCACAT CATTTCTCAA  240
AATATAATAA AGACCATGTG AAATTTAGCG AAATTGCGTG  280
AGAGAACTGT CCAAACATAG GTTAAGGTTA TATGACACCT  320
TTTTCCACCA TACAGTTGCT AAAATGTAGG AATATAAA    358
```

FIG. 1. Nucleotide sequence of a TR-1 element associated with the knob region of maize chromosome 9. The DNA segment between nucleotides 26 and 56 in the TR-1 element shares 64% homology with the conserved segment between nucleotides 122 and 157 of a 180-bp repeat.

presumably as a result of a point mutation. The 0.6-kb *Eco*RI fragment (accession number AF071125) is composed of two truncated inverted segments of TR-1 that can form fold-back DNA. These repeats have a 40-bp unrelated sequence between them. Sequence analysis of the ends of the 6.5-kb *Hae*III fragment revealed that they also are inverted relative to each other. However, adjacent copies of TR-1 elements at each end of the 6.5 kb *Hae*III segment have the same polarity relative to one another.

A detailed restriction map was constructed for the entire cosmid 9 as well as specifically for the 6.5-kb *Hae*III stretch of TR-1 elements (Fig. 2) with the help of a technique based on partial digestion of cosmid clones followed by Southern hybridization with labeled oligonucleotides complementary to the ends of the cloned DNA fragment (see *Materials and Methods* for details). The 6.5-kb *Hae*III fragment contains 18 *Eco*RI restriction sites and 1 *Nde*I site. The differences in length of the *Eco*RI fragments enabled us to place the 0.7-kb dimer of the TR-1 repeat at the position between the fourth and fifth *Eco*RI sites and the 0.6-kb fragment between the eighth and ninth *Eco*RI sites. The 6.5-kb tandem repeat consists of 18 or 19 copies of TR-1 that are divided into two groups of almost equal size, with the point of inversion within the 0.6-kb *Eco*RI fragment. The data on restriction mapping and DNA sequence analysis suggest that the 6.5-kb *Hae*III element is composed of two stretches of TR-1 repeats that are inverted and capable of forming a fold-back DNA sequence (FB-1).

A second stretch of TR-1 elements was found in the same cosmid clone 9 as a part of a 1.2-kb *Nde*I subfragment (Fig. 2). This *Nde*I subfragment is separated from the 6.5-kb *Hae*III subfragment by an ≈2.2-kb stretch. Sequence analysis of this 1.2-kb *Nde*I DNA fragment (accession no. AF071127) revealed that it is composed of two adjacent copies of TR-1 oriented in the same direction, one of which is truncated. The two adjacent TR-1 elements in this 1.2-kb *Nde*I fragment are flanked by

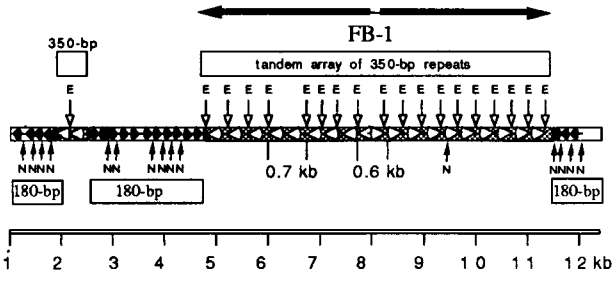

FIG. 2. A molecular map of the knob DNA segment with 180-bp and TR-1 tandem repeats from cosmid 9. The FB-1 element is composed of two inverted arrays of TR-1 elements (indicated by two black arrows) and is flanked by 180-bp repeats. Dashed boxes with clear arrows represent blocks of tandem arrays of TR-1 elements, and clear boxes with black short arrows represent blocks of tandem arrays of 180-bp repeats with arrowheads indicating individual repeats and their orientation. E, *Eco*RI site; N, *Nde*I site.

```
                1<--knob-180-bp -178                83--  TR-1-->
(A)  -TCGAAAAATGGGGGTTGTGTGGCCAC- CTGTATGGTA -TTAGAGTACAACTATTGGATGAAG-

                              TR-1-->357  69-knob-180-bp-->
(B)  -TATAGTTGCTAAAAAGTTGGAATATAA--CACCTACGGATTTTTGACCAAGAAATGGACCCA-

                     knob-180-bp-- >8                                    191-->TR-1
(C)  -CCTCTGGTCGATGATCAATGGCCACAC- ACACATATAAACTTAAACTCATATTGATGTGTTTA--TGTCCACCCAAGTAAAACA-

                        TR-1<-----<------- 349       99 <--knob-180-bp
(D)  -AAAGCTGATCTATTGTTTTGAATTCTTTATATTCC-AACC-GACCATTTCCAGGTCATAAA-
```

FIG. 3. The integration sites of TR-1 elements into 180-bp repeats. All junctures in TR-1 as well as in 180-bp (bold) repeats are different. In three cases, *A*, *C*, and *D*, there are short stretches of unrelated DNA sequences (underlined) that separate the TR-1 from 180-bp repeats.

180-bp repeats. Almost all copies of these 180-bp repeats and TR-1 elements in this region are truncated or have deletions. This observation indicates that significant molecular rearrangements occur on insertions of one type of tandem repeat into arrays of the other type.

The ends of these two blocks of TR-1 elements, FB-1 (the 6.5-kb *Hae*III fragment) and the 530-bp dimer of TR-1 elements, are flanked by stretches of 180-bp repeats. All junction sites differ in both the TR-1 elements and the 180-bp repeats (Fig. 3). The two blocks of TR-1 elements are separated by a stretch of 180-bp repeats ≈2.2-kb long. Sequence analysis indicates that the structure and polarities of these two elements, the TR-1 and the 180-bp repeats, may be rearranged significantly at juncture sites.

The TR-1 element has no homology to any sequence in GenBank and does not cross hybridize to the 180-bp knob repeat. However, dot matrix analysis enabled the identification of two short stretches of DNA sequences that have some level of homology between the 180-bp and TR-1 repeats. The longest region of homology within the TR-1 element is ≈31 bp long (between nucleotides 26 and 56) and shares 64% homology with the segment between nucleotides 122 and 157 of the 180-bp repeat. The second region within TR-1 is 12 bp long between nucleotides 61 and 72 and shares 76% homology with the segment between nucleotides 136 and 148 of the 180-bp repeat. The region between nucleotides 121 and 157 within the 180-bp repeat was identified earlier by Dennis and Peacock (17) as a conserved segment of the 180-bp repeat isolated from maize, teosinte, and *Tripsacum*. The presence of these regions of homology may indicate that the TR-1 element evolved from a 180-bp ancestral repeat as the result of a duplication and subsequent divergence.

Genomic Characterization of TR-1 Elements. Southern blot hybridization of labeled TR-1 DNA to maize genomic DNA digested with different restriction enzymes revealed two patterns of band distribution typical for tandemly arranged DNA sequences (Fig. 4). For example, restriction enzymes *Eco*RI, *Alu*I, *Rsa*I, and *Sau*3A, which have recognition sites within the TR-1 elements, produce a ladder-like banding pattern consisting of monomers and oligomers of TR-1 elements. The *Nde*I, *Taq*I, and *Hae*III restriction enzymes have no recognition sites within the TR-1 repeats, and, as a result, they produce one major group of bands corresponding to high molecular weight DNA fragments >10–20 kb long. These data support a tandem array organization of TR-1 elements.

Restriction Fragment Fingerprinting of the TR-1 Elements in Individual Maize Chromosomes. DNA samples of oat–maize chromosome addition lines for maize chromosomes 2, 3, 4, 7, 8, and 9, respectively, were cut with *Hae*III and fractionated in an agarose gel closed homogeneous electric field

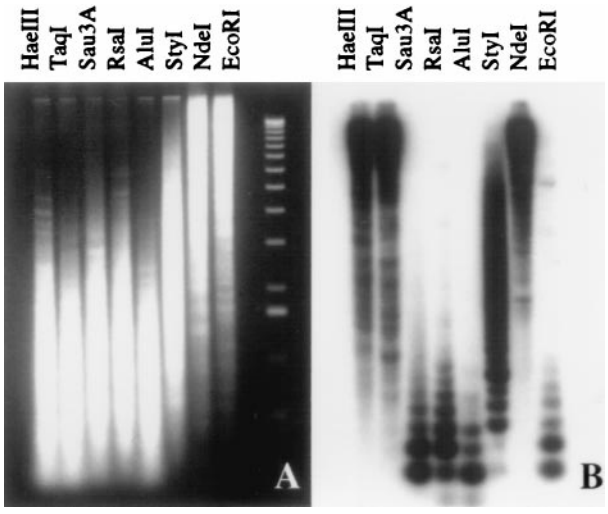

FIG. 4. Hybridization of the TR-1 element to a blot panel of maize genomic DNA (Seneca 60) digested with different restriction enzymes. A typical ladder-like pattern of bands hybridizing to TR-1 is seen in lanes with DNA cut by *Eco*RI, *Alu*I, *Rsa*I, and *Sau*3A, the restriction enzymes that have a recognition site within TR-1. DNA samples cut with *Hae*III, *Taq*I, and *Nde*I, which have no recognition sites within TR-1, reveal maximum hybridization signal with DNA fragments larger than 10–20 kb. *Sty*I produced an intermediate ladder-like pattern of hybridization.

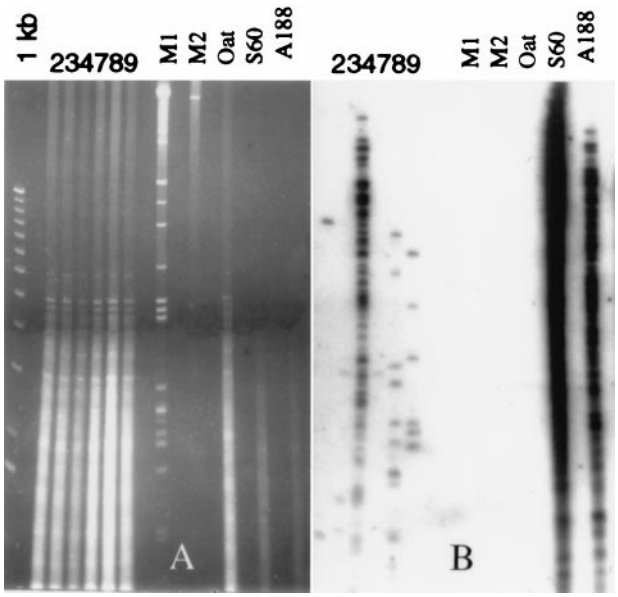

FIG. 5. Hybridization of the TR-1 element to a DNA blot panel of oat–maize chromosome addition lines revealed a high level of polymorphism in copy number and restriction DNA fragment pattern among maize chromosomes and also between two maize lines, Seneca 60 and A188. Plant genomic DNA was cut by *Hae*III restriction enzyme. 1kb, molecular weight 1-kb marker ladder; 2, 3, 4, 7, 8, and 9, oat–maize addition lines with corresponding maize chromosomes; M1, mixture of λ DNA cut with *Hin*dIII and *Pst*I; M2, λ monomer (50 kb and dimer 100 kb); Oat, oat DNA from parental line Starter 1; S60 and A188, DNA of two maize lines (Seneca 60 and A188).

(CHEF)-pulsed field electrophoresis system (Fig. 5). Southern blot hybridization with labeled TR-1 DNA revealed a unique pattern of DNA fragments for each tested maize chromosome. Only a few *Hae*III bands are seen for chromosomes 2, 3, and 7; 6 and 10 bands are seen for chromosomes 9 and 8, respectively; and a large number of fragments (≈30–40) may be seen for chromosome 4. The *Hae*III fragments that hybridize with the TR-1 element varied in size from 1 kb up to 50–70 kb. Summing the lengths of the TR-1 positive *Eco*RI fragments in each of the maize chromosomes provides a rough estimate of the sizes of the chromosomal segments occupied by TR-1 elements in each chromosome. Assuming that each band represents one DNA fragment, the sum of the restriction fragments indicates that the overall size of DNA regions occupied by TR-1 elements varies greatly. For example, chromosome 3 exhibits 4 kb of TR-1 elements whereas chromosome 4 contains ≈400 kb of TR-1 elements.

A large variation in banding patterns and copy numbers of TR-1 elements was observed between the maize varieties Seneca 60 and A188 (Fig. 5). Hybridization of labeled TR-1 DNA with a series of diluted samples of genomic DNA and TR-1 DNA revealed that the TR-1 elements may comprise ≈2% of the total maize genome in Seneca 60 and that the proportion of TR-1 elements may vary significantly in different maize lines (data not shown). No cross hybridization was detected with oat DNA.

Comparative *in Situ* Hybridization of the 180-bp and TR-1 Repeats. Pachytene chromosomes of Seneca 60 was used for *in situ* hybridization analysis because this was the maize donor parent used in the production of the maize chromosome addition lines in oat. Knobs are readily visible in four pachytene chromosomes of this line. They are in the distal half of one of the chromosome arms on chromosomes 4 and 5 and at the end of the short arms of chromosomes 6 and 9 (13). In spite of the fact that all four knob-carrying chromosomes were identified easily, discrimination between chromosomes 4 and 5 was complicated as the result of partial destruction of chromosome morphology during squashing and denaturation steps. Chromosomes 1, 2, 3, 7, 8, and 10 could not be identified because of the lack of any reliable cytological markers. The 180-bp repeat and the TR-1 element were labeled with different fluorescent dyes, and *in situ* hybridization was performed simultaneously with both labeled probes on the same microscopic slide (Fig. 6 *A–C*).

The 180-bp repeat (green in Fig. 6) revealed hybridization to the single cytologically visible knobs on chromosomes 5, 6, and 9 (Fig. 6). No detectable hybridization was found with the large knob on chromosome 4 (Fig. 6*B*). In addition, several weak hybridization sites were found on different chromo-

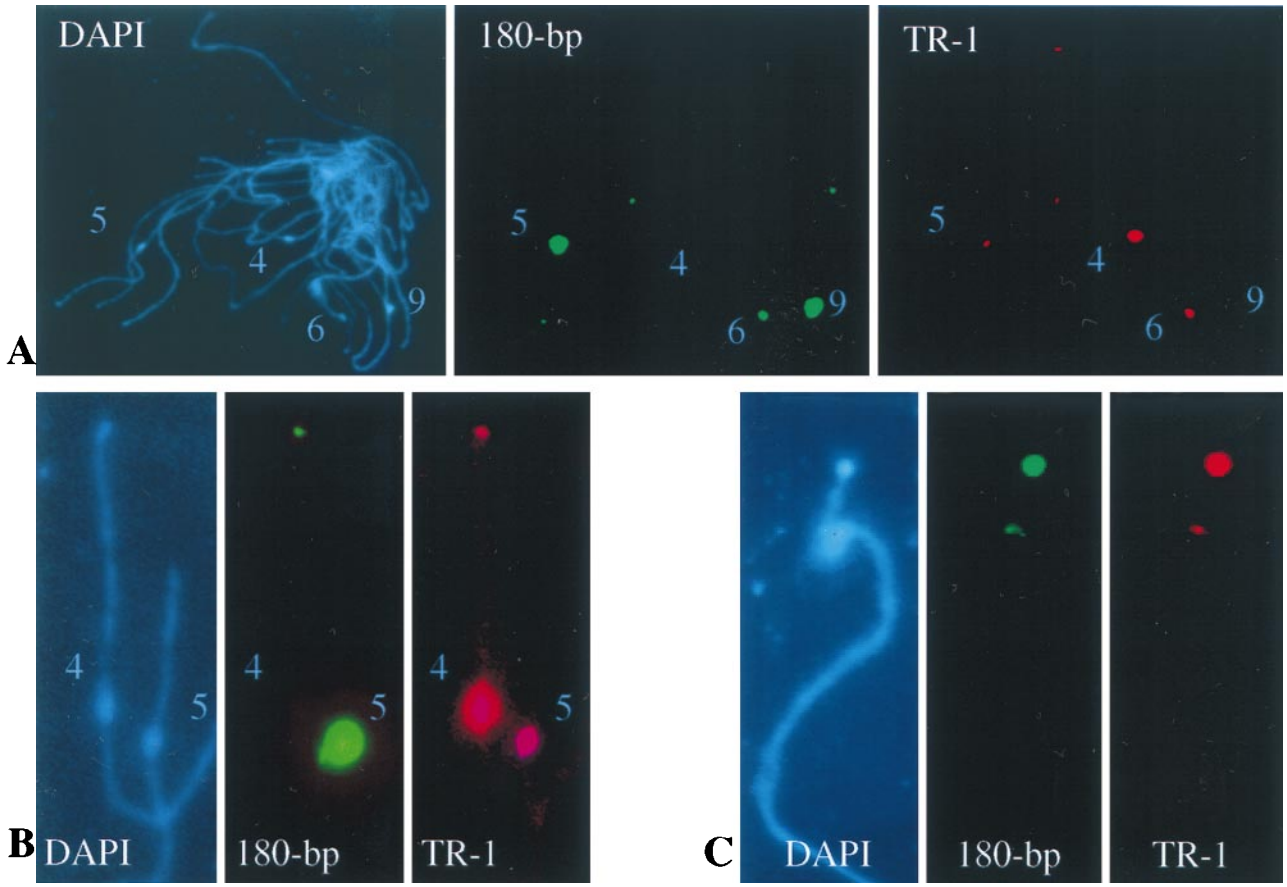

FIG. 6. *In situ* hybridization of the TR-1 and 180-bp repeats to pachytene chromosomes of maize (Seneca 60). (*A*) The overall view of pachytene chromosomes stained with 4′,6-diamino-2-phenylindole (DAPI), the 180-bp repeat (green), and the TR-1 repeat (red) fluorescent images. The 180-bp repeats reveal strong hybridization signal to large knobs on chromosomes 5, 6, and 9, and the TR-1 elements reveal strong hybridization to the large knob on chromosome 4. Several additional small clusters of 180-bp repeats and TR-1 elements may be found in different sites on the chromosomes. (*B*) Distribution of hybridization signals over chromosomes 4 and 5. Overall view of 4′,6-diamino-2-phenylindole-stained chromosomes 4 and 5 (DAPI), the 180-bp repeat (green), and the TR-1 element (red) fluorescent images. The 180-bp repeat is detected in the large knob of chromosome 5 but not in the large knob of chromosome 4. However, there is a small cluster of 180-bp repeats at the terminus of chromosome 4. The TR-1 repeat is detected in both large knobs and in the small cluster at the terminus of chromosome 4. (*C*) Distribution of hybridization signals over chromosome 6. The overall view of 4′,6-diamino-2-phenylindole-stained chromosome 6 (DAPI), the 180-bp repeat (green), and the TR-1 element (red) fluorescent images. The 180-bp repeat and TR-1 element form two clusters: a big one in the knob at the terminus of the short arm and a small one probably in the first chromomere of the satellite of chromosome 6.

somes. One weak hybridization site was found at the end of the presumably long arm of chromosome 4 (Fig. 6*B*), and a small cluster was found at the chromomere flanking the nucleolus organizer region on the satellite of chromosome 6 (Fig. 6*C*). Two small clusters were found on two different unidentified chromosomes, one at the end of one chromosome and another in the subtelomeric region of another chromosome, possibly chromosome 8 (data not shown). When possible, we compared our *in situ* hybridization observations to our estimates of copy number of 180-bp repeats in different maize chromosomes as determined by Southern blot hybridization of knob 180-bp repeats with a blot panel of oat–maize chromosome addition lines (19).

The TR-1 elements (red in Fig. 6) revealed strong hybridization with the cytologically visible knob on chromosome 4 and weak hybridization to the end of the short arm of chromosome 4. The latter coincides with the hybridization site of the 180-bp repeat (Fig. 6*B*). The knob on chromosome 5 hybridizes with the TR-1 repeat as well as the 180-bp repeat (Fig. 6*B*). The prominent knob at the terminus of the satellite on the short arm of chromosome 6 (Fig. 6*C*) has one relatively strong hybridization site with the TR-1 repeats and a second, weak hybridization cluster located on the heterochromatic block at the nucleolus organizer. These two sites coincide with hybridization sites with 180-bp repeats (Fig. 6*C*).

A weak hybridization signal on chromosome 9 was detected with the TR-1 element; this observation is consistent with the weak Southern blot hybridization signal of the TR-1 repeat to genomic DNA from the chromosome 9 addition line (Fig. 5). Two more weak hybridization sites were found on two different unidentified chromosomes. We assume that one of them may be on chromosome 8, which, after chromosome 4, was second in the intensity of Southern blot hybridization of TR-1 repeat with genomic DNA (Fig. 5). These results demonstrate that the TR-1 element is present in knobs of maize chromosomes in different proportions relative to the 180-bp repeat. At least one large knob on chromosome 4 is composed mainly of TR-1 tandem repeats whereas other knobs either are composed mostly of 180-bp repeats or are composed of both types of repeats in different proportions.

DISCUSSION

New Knob-Specific Tandem Repeat. The oat–maize chromosome addition lines in which an individual maize chromosome is retained in an oat genome background provide a unique opportunity to study the structure and composition of specific regions of individual maize chromosomes (25, 26). Recently, we isolated 23 cosmid clones containing knob DNA from maize chromosome 9 (19). Restriction and sequence analysis of these knob DNA segments revealed that the tandem arrays of 180-bp repeats described by Peacock *et al.* (16) are interrupted by insertions of retrotransposable elements. In one of those 23 cosmid clones, we identified a tandem repeat sequence (TR-1). Southern blot hybridization of labeled TR-1 to a blot panel of six maize chromosome addition lines revealed polymorphism in copy number and restriction fragment patterns for TR-1 containing fragments among different maize chromosomes. Polymorphism and copy number variation also were detected for different maize lines.

Comparative *in situ* hybridization of the TR-1 and 180-bp knob repeats to maize pachytene chromosomes revealed that these two types of repeats may be present in some knobs simultaneously. However, some knobs are composed predominantly of either TR-1 or 180-bp repeats. A number of small clusters of these repeats were identified in other sites along the maize chromosomes. In one maize line, Seneca 60, the large cytologically detectable knob on chromosome 4 shows strong *in situ* hybridization with TR-1, and this agrees with the high intensity of Southern blot hybridization of TR-1 to DNA of the maize chromosome 4 addition line. In contrast, the 180-bp repeats gave no detectable *in situ* hybridization with the large knob on chromosome 4. Both repeats, 180-bp and TR-1, hybridize with a second small knob at the end of the short arm of chromosome 4. Cytologically, this site is practically undetectable as heterochromatin. Seven sites of 180-bp repeats and eight of TR-1 were detected reliably in chromosomes of maize line Seneca 60. Five of these clusters contain both 180-bp and TR-1 repeats. In some chromosomes, there are only limited numbers of copies of 180-bp repeats or TR-1 elements, but they can be detected by Southern blot hybridization. In chromosomes 2, 3, and 7, we could identify one or a few bands cross-hybridizing with the 180-bp repeats (19) or with TR-1 elements (Fig. 5) but could not identify corresponding sites by *in situ* hybridization on pachytene chromosomes.

Our observations that some knobs may be composed mostly of TR-1 elements whereas others are composed of 180-bp repeats differ from the conclusion of Dennis and Peacock (17) that all knobs contain 180-bp repeats. We believe that these differences reflect the natural variation in knob numbers and composition among different maize lines. Comparative analysis of these two clustered repeated sequences, TR-1 and 180-bp, in maize lines of different origin should show their distribution along the maize chromosomes and may aid in understanding the evolution of maize.

Genetic effects associated with knob DNA have been attributed mostly to the 180-bp repeat. Our work identified a second family of tandem repeats (TR-1) associated with knobs in maize and revealed a complex structure of knob DNA blocks. We showed also that knobs contain copies of different retrotransposable elements (19), allowing us to suggest that each knob may have a specific organization and composition. This complexity needs to be taken into account when explaining different genetic effects associated with knobs.

Arrangement of the TR-1 and 180-bp Repeats. The maximum stretch of TR-1 tandem repeats isolated and sequenced in this research is ≈6.5 kb. However, most TR-1 elements occur in longer tandem arrays. Southern blot hybridization indicates that a major portion of TR-1 DNA fragments in genomic DNA digested with such restriction enzymes as *Hae*III, *Taq*I, or *Nde*I have sizes >20 kb (Fig. 4), and, in some cases, they may be as long as 70 kb (Fig. 5). Each of these three restriction enzymes cut the 180-bp tandem arrays essentially into monomeric units (19). Thus, these long stretches of TR-1 elements are most likely free of 180-bp repeats. In some other cases, stretches of TR-1 repeats are mixed with stretches of 180-bp tandem repeats. In the case of cosmid 9, which originates from chromosome 9, two stretches of TR-1 elements are inserted into a tandem array of 180-bp repeats. One of them is ≈6.5 kb long, containing 18 copies of TR-1, and the other is composed of two truncated copies of TR-1. Sequence analysis of juncture regions revealed that all juncture points in both the TR-1 and 180-bp repeats are different (Fig. 3). It is interesting that a short stretch of the TR-1 elements and a stretch of 180-bp repeats within the 1.3-kb *Nde*I fragment are composed of truncated copies of these repeats or copies with internal deletions. This suggests that the process of integration of these two families of tandem repeats may be accompanied by local sequence rearrangements and deletions.

Fold-Back Segments of TR-1 Elements. We found that the 6.5-kb *Hae*III fragment from cosmid 9 consists of two inverted stretches of TR-1 elements. These data indicate that TR-1 elements are capable of forming fold-back DNA segments, which are characteristic elements of some transposons (32–34). In contrast to TR-1 elements, the 180-bp repeats have the same polarities in all DNA segments studied (19). The only exception we found was associated with the region of integration of TR-1 elements within the tandem array of 180-bp repeats (Fig. 2). A relatively short, 2.2-kb stretch of a tandem array of 180-bp repeats is flanked by the FB-1 element and a 530-bp dimer of

TR-1 elements. This block of 180-bp repeats is inverted relative to the arrays of 180-bp repeats, which flank the two blocks of TR-1 repeats (Fig. 2).

Knobs as Megatransposons. By combining studies across different maize lines, 23 cytologically distinguishable locations of knob DNA have been identified in maize chromosomes (5, 10, 16, 17). These data, however, cannot reflect all knob sites because, most likely, only the largest knobs actually were detected. Our data show high variation in copy number of the TR-1 elements and the 180-bp knob repeats in different chromosomes. Moreover, our data on *in situ* and Southern blot hybridization indicate that the actual number of knobs or knob sites in the maize genome may be at least twice the number of cytologically detectable knobs.

A remaining question is: Does the knob number variation between different varieties of maize result from local copy number variation of 180-bp repeats or TR-1 elements within any particular knob, or can knobs be transposed from one place in a chromosome to another as a unit? In spite of the fact that knobs are considered as stable chromosomal structures characteristic for each maize line, there is at least one example of genetic instability associated with knobs that is characteristic for transposable elements. Chromosome breakage, deletions of knob regions, or loss of knobbed chromosome may occur at the second microspore division with detectable frequencies in maize lines with two or more B chromosomes (28). In addition to this, our finding that TR-1 elements are capable of forming fold-back DNA sequences indicates similarities of this knob-associated tandem repeat with fold-back elements of giant transposons in *Drosophila* (29–31). *Drosophila* giant transposons are composite elements cytologically visible in larval polytene chromosomes as one or more bands. Their termini consist of fold-back sequences that are themselves transposable and composed largely of a regular 155-bp repeat. The structure of the transposon itself is variable, and regions within it may be lost. These correlations, in conjunction with the facts on knob polymorphisms (5, 10, 16, 17) and instability (28), lead us to propose a hypothesis that maize knobs may be considered as complex megatransposons, and knob polymorphism may be explained as the result of molecular rearrangements and transpositions of entire knob regions or their parts.

The reconstruction of the physical structure and composition of knob regions from different maize chromosomes and from different maize lines may shed light on the nature of knob DNA variability. Oat–maize chromosome addition lines provide a unique opportunity to approach this problem and study the organization of knob regions in individual maize chromosomes.

This is a joint publication of the University of Minnesota and U.S. Department of Agriculture–Agricultural Research Service and is Paper No. 981130052 in the Scientific Journal Series, Minnesota Agricultural Experiment Station. Mention of trademark or proprietary product does not constitute a guarantee or warranty by the University of Minnesota or the U.S. Department of Agriculture–Agricultural Research Service and does not imply approval over other products that also may be suitable. This research was funded in part by Grant. 96–35300-3775 of the U.S. Department of Agriculture National Research Initiative Competitive Grants Program.

1. McClintock, B. (1929) *Science* **69,** 629.
2. McClintock, B. (1930) *Proc. Natl. Acad. Sci. USA* **16,** 791–796.
3. Reeves, R. G. (1944) *Genetica (The Hague)* **29,** 141–147.
4. Longley, A. A. (1952) *Bot. Rev.* **18,** 399–412.
5. McClintock, B., Kato, Y. & Bluemenshein, A. (1981) *Chromosome Constitution of Races of Maize* (Colegio de Postgraduados, Chapingo, Mexico).
6. Welhausen, E. J. & Prywer, C. (1954) *Agron. J.* **46,** 507–511.
7. Mohamed, A. H. & Shoeb, E. A. (1965) *Can. J. Genet. Cytol.* **7,** 388–393.
8. Moll, R. H., Hansen, W. D., Levings, C. S. & Ohta, Y. (1972) *Crop Sci.* **12,** 585–589.
9. Rakha, F. A., El-Metainy, A. Y. & Sabrah, N. S. (1978) *Alexandria J. Agric. Res.* **26,** 153–163.
10. Chughtai, S. R. & Steffensen, D. M. (1987) *Maydica* **32,** 171–187.
11. Chughtai, S. R. & Steffensen, D. M. (1987) *Maize Genet. Coop. Newslett.* **61,** 98–99.
12. Rayburn, A. L., Price, H. J., Smith, J. D. & Cold, J. R. (1985) *J. Bot.* **72,** 1610–1617.
13. Laurie, D. A. & Bennett, M. D. (1985) *Heredity* **55,** 307–313.
14. Rhoades, M. M. (1978) in *Maize Breeding and Genetics*, ed. Walden, B. D. (Wiley, New York), pp. 641–672.
15. Rhoades, M. M. & Dempsey, E. (1973) *J. Hered.* **64,** 13–18.
16. Peacock, W. J., Dennis, E. S., Rhoades, M. M. & Pryor, A. J. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 4490–4494.
17. Dennis, E. S. & Peacock, W. J. (1984) *J. Mol. Evol.* **20,** 341–350.
18. Viotti, A., Privitera, E., Sala, E. & Pogna, N. (1985) *Theor. Appl. Genet.* **70,** 234–239.
19. Ananiev, E. V., Phillips, R. L. & Rines, H. W. (1998) *Genetics*, in press.
20. Longley, A. A. (1938) *J. Agric. Res.* **56,** 177–195.
21. Longley, A. A. (1939) *J. Agric. Res.* **59,** 475–490.
22. Brown, W. L. (1949) *Genetics* **34,** 524–536.
23. Ibrahim, M. A. (1960) *Genetics* **45,** 811–817.
24. Bianchi, A., Ghatnekar, M. V. & Ghindoni, A. (1963) *Chromosoma* **14,** 601–617.
25. Riera-Lizarazu, O., Rines, H. W. & Phillips, R. L. (1996) *Theor. Appl. Genet.* **93,** 123–135.
26. Ananiev, E. V., Riera-Lizarazu, O., Rines, H. W. & Phillips, R. L. (1997) *Proc. Natl. Acad. Sci. USA* **99,** 3524–3529.
27. Sambrook, J., Fritsch E. F. & Maniatis, T. (1989) in *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab. Press, Plainview, NY) pp. 1.60–1.62.
28. Rhoades, M. M. & Dempsey, E. (1972) *Genetics* **71,** 73–96.
29. Ising, G. & Block, K. (1984) *Mol. Gen. Genet.* **196,** 6–16.
30. Chia, W., McGill, S., Karp, R., Gubb, D. & Ashburner, M. (1985) *Nature (London)* **316,** 81–83.
31. Lovering, R., Harden, N. & Ashburner, M. (1991) *Genetics* **128,** 357–372.