Molecular Characterization of a Family of Tandemly Repeated DNA Sequences, TR-1, in Heterochromatic Knobs of Maize and Its Relatives

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ABSTRACT

Two families of tandem repeats, 180-bp and TR-1, have been found in the knobs of maize. In this study, we isolated 59 clones belonging to the TR-1 family from maize and teosinte. Southern hybridization and sequence analysis revealed that members of this family are composed of three basic sequences, A (67 bp); B (184 bp) or its variants B* (184 bp), 2/3B (115 bp), 2/3B* (115 bp); and C (108 bp), which are arranged in various combinations to produce repeat units that are multiples of ~180 bp. The molecular structure of TR-1 elements suggests that: (1) the B component may evolve from the 180-bp knob repeat as a result of mutations during evolution; (2) B* may originate from B through lateral amplification accompanied by base-pair changes; (3) C plus A may be a single sequence that is added to B and B*, probably via nonhomologous recombination; and (4) 69 bp at the 3’ end of B or B*, and the entire sequence of C may be removed from the elements by an unknown mechanism. Sequence comparisons showed partial homologies between TR-1 elements and two centromeric sequences (B repeats) of the supernumerary B chromosome. This result, together with the finding of other investigators that the B repeat is also fragmentarily homologous to the 180-bp repeat, suggests that the B repeat is derived from knob repeats in A chromosomes, which subsequently become structurally modified. Fluorescence in situ hybridization localized the B repeat to the B centromere and the 180-bp and TR-1 repeats to the proximal heterochromatin knob on the B chromosome.

Knobs are blocks of heterochromatin present on chromosomes of maize and its relatives, teosinte and Tripsacum, and are most conspicuous at prophase of the first meiotic division. According to the classification of Iltis and Doebley (1980), the genus Zea can be divided into two sections, Luxuriantes and Zea. The former contains three species, Z. diploperennis, Z. perennis, and Z. luxurians, while the latter contains a single species, Z. mays, which can be further divided into four subspecies, ssp. mays, ssp. mexicana, ssp. parviglumis, and ssp. huehuetenangensis (Doebley 1990). Z. mays ssp. mays is the cultivated maize, while all other wild taxa in the genus Zea are known as teosinte. The number, size, and chromosomal position of knobs vary among maize, teosinte, and Tripsacum; knob constitutions, therefore, provide useful information on phylogenetic relationships and the origin of cultivated maize (McClintock et al. 1981; Kato and Lopez 1990).

Several genetic effects have been reported to be associated with the knobs of maize. Abnormal 10 (Ab10) is an unusual type of chromosome that differs from the normal 10 (N10) by having an extra segment of chromatin at the end of the long arm, on which are situated a large knob called K10 and three small knobs (Rhoades 1978). When Ab10 is present, K10 and knobs on other chromosomes form neocentromeres that pull knobs toward the spindle poles ahead of the true centromeres (Rhoades 1942; Longley 1945; Yu et al. 1997), so that in knobbed/knobless heterozygotes there is preferential recovery, or meiotic drive, of knobbed chromosomes and genes linked to them (Rhoades and Dempsey 1966). Knobs also affect the frequency and position of genetic recombination (Rhoades 1978). Recently, Buckler et al. (1999) proposed that meiotic drive plays an important role in evolution of the maize genome. Rhoades and Dempsey (1973) showed that when two or more supernumerary B chromosomes, which are composed mainly of heterochromatin, are present in a maize plant, the interaction of Bs and knobs leads to delayed replication of knobs at the second microspore division and, consequently, to chromosome breakage and chromatin loss.

Two families of tandemly repeated DNA sequences, the 180- and 350-bp (TR-1) repeats, have been found in maize knobs. The former consists of a major component with a sequence 180 bp long and a minor 202-bp variant derived from the 180-bp repeat by a duplication of 22 bp after position 24 (Peacock et al. 1981; Dennis and...
The 180-bp repeats are present in maize, teosinte, and Tripsacum, but not in Coix and Sorghum (Dennis and Peacock 1984). TR-1 elements were originally isolated from an oat–maize addition line for chromosome 9 of maize cv. Seneca 60 (Ananiev et al. 1998b). The occurrence of TR-1 elements in other maize lines and in teosinte and Tripsacum has not been extensively investigated.

Ananiev et al. (1998b) found two regions of 31 and 12 bp that are homologous between the 180-bp and TR-1 repeats and proposed that TR-1 may have evolved from the 180-bp repeat as the result of a duplication and subsequent divergence. They showed, by fluorescence in situ hybridization (FISH), that in maize cv. Seneca 60 the two families of tandem repeats may be present in some knobs simultaneously, while other knobs are composed entirely of TR-1 elements or 180-bp repeats. Hiatt et al. (2002) found that both families of repeats show neocentromere activity in the presence of Ab10, but the activity of TR-1 elements is higher than that of 180-bp repeats. Alfenito and Birchler (1993) compared the nucleotide sequence of the 180-bp repeat with a centromeric repeat of the B chromosome, ZmBs (also named B repeat), and found a 72% homology between the two sequences over a 90-bp stretch. Recently, Page et al. (2001) found that the 180-bp repeat is also homologous to the centromeric sequence of maize chromosome 4 (Cent4). However, homology of TR-1 to the B centromere and Cent4 has not been studied.

In this study, we isolated 59 TR-1 elements from maize and teosinte and studied the molecular structure of these elements by sequence analysis, Southern hybridization, and FISH. Our results showed that the structure of TR-1 elements is more complex than previously understood. We propose a hypothesis for the origin of TR-1 elements and suggest an evolutionary relationship between knob repeats and the centromeric repeat of B chromosomes.

MATERIALS AND METHODS

Plant material: The plant material used in this study is listed in Table 1. All taxa except Sorghum bicolor and Coix lacryma-jobi possess 180-bp repeats (Dennis and Peacock 1984). Seneca 60 and KYS are maize stocks that have been shown to have TR-1 elements (Ananiev et al. 1998b; Chen et al. 2000). Maize stocks K10 and BMS contain Ab10 and B chromosomes, respectively.

Southern hybridization: Total genomic DNA (2–5 μg per lane, depending on the length of probes) isolated from young leaves of Dellaporta (1994) was digested with EcoRI and electrophoresed on a 1.2% agarose gel. Blotting was conducted as described by Kreike et al. (1990). A monomer of a TR-1 element, ZmKR350-1 (Chen et al. 2000), was labeled with digoxigenin-11-dUTP (Roche Molecular Biochemicals, Indianapolis) by nick translation and hybridized to membrane-bound genin-11-dUTP (Roche Molecular Biochemicals, Indianapolis) by nick translation or polymerase chain reaction (PCR). The hybridization mixture consisted of 50% formamide, 2× SSC (0.15 M sodium chloride, 0.015 M sodium citrate), 10% dextran sulfate, 0.1% SDS, 5 ng/μl probe DNA, and 1 μg/μl herring sperm DNA. Hybridization was carried out at 37°C overnight, followed by washes in 20% formamide, 0.2× SSC at 42°C for 10 min, in 2× SSC at 42°C for 10 min, and in 2× SSC at room temperature three times for 5 min. To investigate the relative chromosomal locations of two repetitive sequences, probes were hybridized simultaneously to meiotic chromosome spreads. Digoxigenin-labeled probes were detected with fluorescein-conjugated antibodies (Roche), and signals were amplified with fluorescein-conjugated anti-sheep IgG (Vector Laboratories, Burlingame, CA). Biotin-labeled probes were detected with avidin-Texas red (Vector Laboratories). Chromosomes were counterstained with 4′,6-diamidino-2-phenylindole. Slides were visualized under an Olympus AX70 fluorescence microscope with appropriate filter sets. The images were captured using a cooled CCD camera (PXL 1400; Photometrics, Tucson, AZ) and final image adjustments were done with Adobe Photoshop 5.0 software.

RESULTS

Distribution of TR-1 elements in maize and its relatives: Southern hybridization of genomic DNA from maize and its relatives with the TR-1 probe revealed that all stocks of maize and races of teosinte contain the TR-1 element; however, no TR-1 element was detected in the genomes of S. bicolor, C. lacryma-jobi and Tripsacum dactyloides (Figure 1). Moreover, a ladder-like banding pattern consisting of monomers of 180 bp was observed, instead of 350 bp (Ananiev et al. 1998b). The copy number of TR-1 elements varies from one stock to another in maize and teosinte, as revealed by the intensity labeling and hybridized to EcoRI-digested, membrane-bound DNA at 55°C in 5× SSPE, 0.1% SDS; the final wash was done at 55°C in 2× SSPE, 0.1% SDS. Hybridization signals were detected by a chemiluminescence reaction using CSPD (Roche) as substrate.

Cloning and sequencing of TR-1 elements: Genomic DNA isolated from maize and teosinte was digested with EcoRI and electrophoresed on a 1.2% agarose gel. DNA fragments 150–750 bp long (Figure 1) were recovered, ligated to pUC18, transformed into Escherichia coli strain XL1-blue and screened by colony and Southern hybridization using the TR-1 element ZmKR350-1 as a probe. Positive clones were sequenced using an ABI PRISM 377 automatic sequencer (Perkin-Elmer, Norwalk, CT). Sequences were compared to GenBank and ZmDB entries using BLAST software and alignments were made using Laser Gene software (DNASTAR, Madison, WI). Fluorescence in situ hybridization: Maize stocks KYS, BMS (with B chromosomes), and K10 (with Ab10s) were used for FISH. Meiotic chromosomes were prepared according to the method of Chen et al. (1998). TR-1 repeat ZmKR350-1, a dimer of the 180-bp repeat, ZmKR180-2 (Chen et al. 2000), and a modified B repeat, B1.1a (GenBank accession no. AY173950), were used as probes. The modified B repeat differs from the original B repeat in lacking the region that shares homology with the 180-bp repeat (A. Kato, T. Phelps-Durr and J. A. Birchler, unpublished work). The advantage of using the modified B repeat as a probe for the centromere is that the possibility of cross-hybridization between the B repeat and the 180-bp repeat can be avoided. Probes were labeled with digoxigenin-11-dUTP or biotin-16-dUTP (Roche) by nick translation or polymerase chain reaction (PCR). The hybridization mixture consisted of 50% formamide, 2× SSC (0.15 M sodium chloride, 0.3 M trisodium citrate), 10% dextran sulfate, 0.1% SDS, 5 ng/μl probe DNA, and 1 μg/μl herring sperm DNA. Hybridization was carried out at 37°C overnight, followed by washes in 20% formamide, 0.2× SSC at 42°C for 10 min, in 2× SSC at 42°C for 10 min, and in 2× SSC at room temperature three times for 5 min. To investigate the relative chromosomal locations of two repetitive sequences, probes were hybridized simultaneously to meiotic chromosome spreads. Digoxigenin-labeled probes were detected with fluorescein-conjugated antibodies (Roche), and signals were amplified with fluorescein-conjugated anti-sheep IgG (Vector Laboratories, Burlingame, CA). Biotin-labeled probes were detected with avidin-Texas red (Vector Laboratories). Chromosomes were counterstained with 4′,6-diamidino-2-phenylindole. Slides were visualized under an Olympus AX70 fluorescence microscope with appropriate filter sets. The images were captured using a cooled CCD camera (PXL 1400; Photometrics, Tucson, AZ) and final image adjustments were done with Adobe Photoshop 5.0 software.
TR-1 Elements in Maize and Teosinte

TABLE 1

Plant material used in this study

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<tr>
<th>Taxon</th>
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<th>Source</th>
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A665 20 NCRPIS
KIS 20 MGCSC
K10 20 MGCSC
BMS with Bs 20 + Bs MGCSC
M03G-11A 20 TARI
Seneca 60 20 P. C. Cheng, SUNY

NTU, National Taiwan University, Taipei, Taiwan; TARI, Taiwan Agriculture Research Institute, Taichung, Taiwan; CIMMYT, International Maize and Wheat Improvement Center, Mexico City, Mexico; NCRPIS, North Central Regional Plant Introduction Station, USDA, Ames, Iowa; MGCSC, Maize Genetics Cooperation Stock Center, Urbana, Illinois; SUNY, State University of New York, Buffalo, NY.

of hybridization signals; Z. diploperennis appeared to have the highest copy number while Z. mays ssp. huehuetenangensis had the lowest copy number among the taxa studied.

Molecular structure of TR-1 elements: A total of 59 TR-1 elements were isolated from maize and teosinte. Analysis of DNA sequences of these elements revealed that they were composed of three basic sequences, A, B (or its variants, see below), and C, which were organized in various combinations to produce repeat units that are multiples of ~180 bp (Figure 2). A and B (or its variants) were present in all TR-1 elements regardless of their lengths; however, the C component was not found in the 180-bp elements. The distribution of various types of TR-1 elements in maize and teosinte is shown in Table 2. Type 3 and type 1 360-bp elements were predominant in the TR-1 family.

The A component is 67 bp long and contains ~70% A + T, and within it are three restriction endonuclease-cutting sites, EcoRI, Sau3AI, and DraI. Cloning of TR-1 elements from EcoRI-digested genomic DNA, therefore, separates the A component into two parts, A1 (36 bp) and A2 (31 bp), located respectively at the 3′ and 5′ ends of TR-1 elements. Some longer TR-1 elements (540 and 720 bp) contain an intact A component internally (Figure 2), as a result of loss of the EcoRI-cutting sequence caused by point mutation or duplication of the nucleotides AATT. Except for clone MEX-1 (GenBank accession no. AY083970), which has a 14-bp deletion at the 5′ end, the sequences of the A component are very conserved, with homologies of >90% both within and between taxa (Table 3).

The B component has two different lengths, 184 and 115 bp, both of which can be further divided into two classes, B and B′ (184 bp) and 2/3B and 2/3B′ (115 bp), according to their nucleotide sequences. Homologies of these sequences within and between taxa are shown in Table 3. The greater variations in B and B′ are caused mainly by differences among the clones from Z. mays ssp. huehuetenangensis and maize stock K10. The first 115 bp of the consensus sequence of B shows closer homology to the sequence of 2/3B (96.5%) than to that of 2/3B′ (91.3%); similarly, the first 115 bp of the consensus sequence of B′ shows closer homology to the sequence of 2/3B′ (95.7%) than to that of 2/3B (88.7%). Although homologies between the consensus sequences of B and B′ and between 2/3B and 2/3B′ are fairly high (90.2 and 93.0%, respectively), B (and 2/3B) differs from B′ (and 2/3B′) at several characteristic positions, such as 1, 6–8, 24, 38, and 42 (Figure 3). One interesting feature of the structural organization of TR-1 is that when B and B′ (2/3B′) are adjacent in the same clone, B is always 5′ to B′ (2/3B′; Figure 2).

The C component is 108 bp long and contains ~67% A + T. Analysis of sequences of five 180-bp TR-1 elements isolated from teosinte showed that they contained A and 2/3B or 2/3B′ but no C (Figure 2). To test the generality of this result, we probed EcoRI-digested genomic DNA from maize and teosinte with oligonucleotides from the three components of TR-1 (Figure 3). The luminograms revealed 180-bp bands in all taxa when oligonucleotides of A or B were used as probes, but not when an oligonucleotide of C was the probe (Figure 4). Two regions of homology were found be-
Figure 2.—Schematic representation of the molecular structure of 59 TR-1 elements isolated from maize and teosinte (GenBank accession nos. AY083937–AY083995). Symbols for restriction sites are as follows: EcoRI (E), Sau3AI (S), DraI (D), RsaI (R), and AluI (A).

A sequence homology search revealed that 78 clones in the ZmDB database contained TR-1 sequences. Sequence analysis showed that 57 of these clones contained exclusively TR-1 sequences while the rest possessed 180-bp knob repeats, segments of coding genes such as bz and zein, or unknown sequences at the ends or in the internal region of TR-1 sequences. Since these clones were isolated by the shotgun method, the presence of nonkob sequences and in some cases 180-bp knob repeats may be the result of DNA rearrangement that occurred during library construction. The inserts of the 78 clones were short (112–829 bp) and only 18 carried the component sequences for a complete TR-1 element: two 180-bp type 1 (accession nos. BH127737 and BZ618680), six 360-bp type 1 (accession nos. BH780746, BZ407231, BZ530342, BH780540, BH773823, and BH787856), four 360-bp type 2 (accession nos. BZ306240, BZ361258, BZ322764, and BZ618935), three 540-bp type 1 (accession nos. BH774018, BH873469, and BZ312807), two 540-bp type 2 (accession nos. BZ376526 and BZ530348), and one 720-bp type 1 (accession no. BZ618681; see Figure 2).

Homology of TR-1 elements to other repeated sequences: The consensus sequence of 360-bp type 1 TR-1 elements (Figure 2) was compared with the 180-bp knob repeat (Dennis and Peacock 1984), two centromeric B repeats, ZmBs and K5 (Alfenito and Birchler 1993; Page et al. 2001), and the centromeric sequence of chromosome 4, Cent4 (Page et al. 2001), and the results are summarized in Figure 5. Two regions of homology were found between the 180-bp repeat and the B component.
TABLE 2

TR-1 elements isolated from maize and teosinte

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*a See Figure 2.

of TR-1. In the 180-bp repeat, the two regions are separated by 26 bp, while in the B component of TR-1, one region is included in the other, suggesting that the 180-bp repeat may contain subrepeats but that the B component of TR-1 does not. Regions of homology were found between ZmBs, K5, Cent4, and all three components of TR-1. In Cent4 and ZmBs, some regions homologous to component C are also homologous to component A, confirming the result from sequence comparison between C and A. Two to three different regions in Cent4 and K5 are homologous to regions that are overlapping in component C or A, suggesting that Cent4 and K5 may contain duplicated segments similar to sequencing in C or A.

**Locations of 180-bp and TR-1 repeats on A chromosomes:** Chromosomal locations of 180-bp and TR-1 repeats in the maize inbred line KYS have been studied previously (Chen et al. 2000). In that study, we found that 180-bp repeats were located in cytologically detectable knobs on 5L, 6S, 6L, 7L, and 9S, as well as at several terminal and interstitial sites where heterochromatin was not apparent. However, only two clusters of TR-1 repeats were observed, one located alone at 4L.89 and the other colocated with 180-bp repeats in the terminal knob (satellite) on 6S. In this study, with the aid of a more sensitive signal-detection device, the cooled CCD camera, we were able to detect more hybridization sites under the same stringency conditions as used previously (Figure 6, A–C). Particularly interesting is the observation of weakly dispersed signals in the large knobs on 5L and 7L when TR-1 element was the probe.

FISH of 180-bp and TR-1 repeats to pachytene spreads of maize stock K10 revealed that the three small knobs on Ab10 contained TR-1 elements and probably other unknown sequences, while the large knobs on Ab10 and other chromosomes consisted predominantly of 180-bp repeats. In the large knobs in which 180-bp and TR-1 repeats coexisted, TR-1 repeats were either clustered in one part of the knob or interspersed with 180-bp repeats and probably also other sequences (Figure 6, D–F). The occurrence of 180-bp and TR-1 repeats in knobs on Ab10 is consistent with the result of Hiatt et al. (2002).

**Relative locations of knob repeats and the B centromere:** The B chromosome of maize consists chiefly of

TABLE 3

Comparison of the consensus sequences of various components of TR-1 in maize and teosinte

<table>
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<tr>
<th>Component</th>
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<th>No. of sequences analyzed</th>
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<th>Sequence homology (%)</th>
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<td>C</td>
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<td>28</td>
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<td>91.0–95.5</td>
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*a One element (GenBank accession no. AY083970) was not involved in the comparison because of deletions in components A and 2/3B'.

TR-1 Elements in Maize and Teosinte
heterochromatic segments, one located adjacent to the terminal centromere (proximal heterochromatin knob) and several on the distal portion of the long arm (distal heterochromatin blocks; RHOADES 1978). Simultaneous FISH of two probes to meiotic spreads of the stock BMS with Bs revealed that at pachynema most signals from the 180-bp repeat were clustered in the proximal heterochromatin knob and the rest were in the most distal heterochromatin block on the long arm of the B chromosome (Figure 6G). When TR-1 was the probe, however, only the proximal heterochromatin knob was weakly labeled (Figure 6H). Superimposition of the images from 180-bp and TR-1 repeats revealed that chromosomal locations of these two repeats were similar (Figure 6I). The modified B repeat also showed two hybridization sites on the B chromosome, a major site in the centromeric region and a minor site adjacent distally to 180-bp repeats on the long arm (Figure 6, J–L). At metaphase I, arrays of the modified B repeat stretched toward the spindle poles, whereas 180-bp repeats lagged behind (Figure 6, M–O), suggesting that the modified B repeat is located in the B centromere but the 180-bp and TR-1 repeats are not.

**DISCUSSION**

TR-1 elements were first isolated from the knob DNA of chromosome 9 of maize cv. Seneca 60 by ANANIEV et al. (1998b). These investigators analyzed nucleotide sequences of seven EcoRI fragments from a cosmid clone containing 180-bp repeats and found that these fragments were monomers or dimers of a sequence of ~180 bp in length. In this study, we isolated 59 clones from EcoRI-digested genomic DNA of several stocks of maize and races of teosinte. Southern blot and sequence analyses revealed that the molecular structure of TR-1 elements is more complex than previously thought. The family of TR-1 elements is composed of three basic sequences, A (67 bp); B (184 bp) or its variants B’ (184 bp), 2/3B (115 bp), and 2/3B’ (115 bp); and C (108 bp). These components are organized into various combinations to produce repeats that are multiples of ~180 bp (Figure 2), a length characteristic of satellite DNA in many plant species (Martinez-Zapater et al. 1986; Grellet et al. 1986; Koukalová et al. 1989, 1995).

The structure discussed above is supported by the finding that the 18 TR-1 elements in the ZmDB database fall nicely into six major types in the TR-1 family. An explanation for the discrepancy between the results of ANANIEV et al. (1998b) and our results may be that the different types of TR-1 element are nonrandomly distributed in the knob DNA, as the seven clones analyzed by ANANIEV et al. (1998b) were isolated from a 12-kb cosmid clone, whereas those obtained by us and in the ZmDB database were from genomic DNA. The data available so far suggest that there seems to be no preferential occurrence of particular types of TR-1 element in maize and teosinte.

The origin of maize has been controversial (DOEBLEY 2001). However, evidence from morphology, genetics, cytogenetics, biochemistry, and molecular biology is accumulating to indicate that teosinte, rather than Tripsacum, is the progenitor of maize (BEADLE 1978; McClintock et al. 1981; Dennis and Peacock 1984; Doebely 1990; Kellogg and Birchler 1993; Buckler and Holtsford 1996). The absence of TR-1 elements in Tripsacum and the high homologies of individual components of TR-1 between maize and teosinte provide additional evidence for the “teosinte hypothesis” (BEADLE 1939).

The nucleotide sequence of the 180-bp knob repeat of maize has been analyzed by Grellet et al. (1986) and Ingham et al. (1993), and both groups of investigators found subrepeats in this satellite sequence. In this study, comparison of the nucleotide sequences of the 180-bp and TR-1 repeats revealed two regions of homology between the 180-bp repeat and the B component of TR-1. In the 180-bp repeat the two regions are separated by 26 bp, while in the B component of TR-1 one region is included in the other (Figure 5). This result suggests that the B component of TR-1 may have originated from the 180-bp repeat by loss of internal repetition caused by mutations during evolution. This hypothesis is supported by the presence of 180-bp repeats but the absence of TR-1 elements in Tripsacum.
sence of TR-1 elements in Tripsacum, a genus from which maize and teosinte have diverged (Kellogg and Birchler 1993; Buckler and Holtsford 1996).

We have shown that in both maize and teosinte the B component (and 2/3B) differs from B’ (and 2/3B’) at several characteristic positions. Furthermore, when B and B’ (2/3B’) are adjacent in the same clone, B is always 5’ to B’ (2/3B’). These observations suggest that B’ (2/3B’) may have originated from B (2/3B) by lateral amplification accompanied by sequence changes prior to the evolutionary divergence of maize and teosinte.

Several findings suggest that C/H11001 may actually be a single repeat sequence that has a length similar to that of B, but which differs from B in nucleotide sequence and base composition and in containing sub-repeats. First, A and C share 67% homology in two regions with a total length of 43 bp. Second, they both have a high A + T content of ~70%. Third, regions in Cent4 and ZmBs that are homologous to C also share homology with A. Fourth, when C and A are adjacent in the same clone, C is always 5’ to A.

On the basis of the arguments given above, we propose a model for the origin of the complex structure of TR-1 elements (Figure 7). In this model, the B component has evolved from the 180-bp repeat. Addition of C/H11001 to B gives rise to the 360-bp type 1 element (Figure 2) that is structurally similar to the 350-bp element isolated by Ananiev et al. (1998b). Lateral amplification of B by a mechanism that would simultaneously induce nucleotide changes followed by the addition of C + A results in the formation of the 540-bp type 1 element. Excision of 69 bp from the 3’ end of B (B’) and the entire sequence of C from 360-bp type 1 and 540-bp type 1 repeats produces 180-bp type 1 and 360-bp type 3 elements, respectively. The 540-bp type 2 element may result from two rounds of lateral amplification of B, addition of C + A, and excision of 69 bp from the 3’ end of the B’ component and of the entire C component. The 720-bp types 1 and 2 and the 540-bp type 3 repeats are composite elements in which the internal EcoRI-cutting site has been lost. The long-range organization of TR-1 elements and their positions relative to the 180-bp repeats and other sequences in knobs have not been established from the data obtained in this study. Cloning of large fragments from knob DNA and analysis of their structure would be necessary to understand the long-range organization.

Several mechanisms have been proposed to explain the expansion and contraction of arrays of tandem repeats and addition of ectopic, nonhomologous sequences to DNA molecules in eukaryotic genomes. Among these, synthesis-dependent strand annealing (Gorbunova and Levy 1997; Pâques et al. 1998), in our opinion, may be most relevant to explain the events proposed in our model. One thing remains unexplained, however: when excision occurs, it always involves the same DNA segment in all clones studied.

Although the last few nucleotides of 2/3B and 2/3B’ differ from those of B and B’ (Figure 3), there is no reason to believe that this would be related to the excision.

The mechanism of neocentromere formation in maize knobs is not fully understood. From a combined FISH and immunocytochemical study, Yu et al. (1997) showed that, unlike standard kinetochores, neocentromeres associate laterally with microtubules, which suggested that they are mobilized on microtubules by the activity of minus end-directed motor proteins. Dawe et al. (1999) and Zhong et al. (2002) further showed that CENPC and CENH3, proteins presumably required for centromere function in maize, are absent on neocentromeres of maize knobs. The work of Dawe and colleagues suggests that the mechanism of neocentromeric activity of maize knobs is different from that of true centro-
meres and probably also from that of neocentromeres found in humans and Drosophila (du Sart et al. 1997; Williams et al. 1998). Recently, Hiatt et al. (2002) found that 180-bp and TR-1 knob repeats differ in their capacity to form neocentromeres and that their mobility is controlled by two different trans-acting factors that are encoded or recruited by different genes in the extra segment of Ab10. They further suggested that the trans-acting factors may be a class of kinesins.

FISH to pachytene spreads showed that the modified B repeat has a major hybridization site in the centromere and a minor site in the most distal heterochromatin block on the long arm of the B chromosome, confirming the result of Alfenito and Birchler (1993). Assuming that the B repeat is located within the functional domain of the B centromere (Kaszás and Birchler 1996, 1998), this result may mean that the B chromosome of maize is dicentric. However, FISH to metaphase I chromosomes revealed that of the two sites containing B repeats, only the major site displays centromeric activity, as revealed by stretching of hybridization signals toward the spindle poles (Figure 6, M–O). Pлатеро et al. (1999) explained that this phenomenon results from the major site being dominant over the minor site in kinetochore formation because it has more copies of centromeric DNA sequences. They further proposed that differences in strength proportional to size might be characteristic of centromeres in general.

In maize, B chromosome nondisjunction and loss of knobbed A chromatin, both occurring at the second microspore division, have been attributed to late replication of the proximal heterochromatin knob on the B chromosome and of knobs on A chromosomes, respectively (Rhoades and Dempsey 1973). From a comparative study of the time of replication of euchromatin and several classes of heterochromatin in the maize genome, Pryor et al. (1980) found that the proximal knob on the B chromosome and knobs on A chromosomes are the latest components to replicate during the S phase of the mitotic cycle. The presence of 180-bp and TR-1
repeats in these knobs (Peacock et al. 1981; Viotti et al. 1985; Ananiev et al. 1998b; this study) suggests that B chromosome nondisjunction and loss of knobbed A chromatin may actually be caused by late replication of the tandem repeats in knobs. Accumulation of tandem repeats in a location closely adjacent to the centromere seems to be selectively advantageous to the maintenance of B chromosome polymorphism in maize.

It has been shown that B chromosome-specific clones isolated from maize and rye share homology with fragments of a variety of sequences, such as retrotransposons, tandem repeats, and coding genes, in the normal chromosome complement (Alfenito and Birchler 1993; Stark et al. 1996; Langdon et al. 2000; Page et al. 2001). This has led investigators to propose that the B chromosome is a conglomerate of DNA fragments from different A chromosomes. Homologies between the B repeat and 180-bp and TR-1 repeats as revealed by sequence comparison (Alfenito and Birchler 1993; this study) suggest that portion of the B centromere is derived from the tandem repeats in knobs, which subsequently become structurally modified as a result of adaptation to a new chromosomal environment. The tandem repeats may move away from their original locations on A chromosomes through: (1) chromosome breakage caused by late replication of knob DNA (Pryor et al. 1980); (2) transposition of tandem repeats themselves, as they could form foldback structures characteristic of mobile elements (Ananiev et al. 1998b; Buckler et al. 1999); or (3) transposition of retrotransposons that are inter-
spersed with arrays of tandem repeats in knobs (Ananiev et al. 1998a).

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LITERATURE CITED


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