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Pinning Down Loose Ends: Mapping Telomeres and Factors Affecting Their Length

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A degenerately repeated sequence, proximal to the telomere heptanucleotide repeat in maize, contains restriction enzyme sites that permit the separation of telomeres from the rest of the chromosomes. Probing with a telomere-specific oligonucleotide revealed genotype-dependent telomere lengths that vary more than 25-fold in maize among the 22 inbreds that have been surveyed. These lengths were found to segregate reproducibly in a recombinant inbred family where 50% of the variation can be accounted for by three loci. The dynamic control over telomere length in maize appears to act rapidly to achieve new genotypically determined telomere lengths in the F_1. Clones of telomere proximal sequences were used to map restriction fragment length loci at the distal ends of eight of 20 chromosome arms.

INTRODUCTION

We have been augmenting a molecular map of the maize genome based on two recombinant inbred families (Burr and Burr, 1991). One of our goals has been to define the ends of the linkage groups. Richards and Ausubel (1988) found that the telomere repeat in a number of higher plants, including maize, was 5′-CCCTAAA-3′. The same sequence has been localized to rye telomeres by in situ hybridization (Schwarzacher and Heslop-Harrison, 1991). Ganal et al. (1991) determined that the C-rich strand consensus repeat for tomato telomeres was CCCT(T/A)AA. We used an oligonucleotide containing four tandem repeats of CCCTAAA to probe maize inbreds in the hope of finding restriction fragment length polymorphisms (RFLPs) that would allow us to map telomeres. We discovered that average telomere length was remarkably polymorphic between maize lines and appeared to be under tight genetic control within a given strain.

Telomeres perform at least two functions: they prevent chromosomes from fusing and they permit the stable replication of chromosome termini. These functions are apparently related to their particular structure and require that they be replicated by specialized processes. DNA polymerases synthesize DNA only in the 5′ to 3′ direction and require an RNA primer to begin. Once the primer is removed, there is no way to replace it. Without some special mechanism of synthesis, chromosome ends would shorten with each round of replication (Watson, 1972). An RNA-dependent DNA polymerase, first found in ciliates (Greider and Blackburn, 1985, 1987), is responsible for extending the telomere repeat G-rich strand distally from the end of the chromosome and the sequence synthesized is determined by an RNA template bound to the enzyme. An RNA primer is then copied from the extended, single-stranded G-rich 3′ end (Zahler and Prescott, 1989). The C-rich strand is presumably filled in by a primer-mediated DNA polymerase more typical of eukaryotic chromosome replication. These steps apparently require additional specific telomere binding proteins (Gottschling and Zakian, 1986; Lustig et al., 1990). A variety of gene products are involved in the synthesis, maintenance, and, probably, degradation of these structures. Telomere length is, therefore, likely to be determined by a number of genes.

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RESULTS

Telomeric termini are not covalently closed and are uniquely sensitive to exonuclease digestion. As shown in Figure 1, the variably sized fragments hybridizing to the $d(CCCTAAA)_4$ oligonucleotide are continually shortened and eventually destroyed by prolonged treatment with BAL 31 nuclease. In contrast, repeated DNA stained with ethidium bromide in this gel showed little degradation due to BAL 31 digestion. Internal sequences complementary to the oligonucleotide probe, also seen in Figure 1, were similarly resistant to degradation. We assume that the oligonucleotide probe recognizes all 20 maize telomeres and that the blur (as opposed to a discrete band) observed on these autoradiograms is due to heterogeneity in the length of the telomeres for a given genotype, as well as polymorphism of the subterminal sequences cleaved by the restriction endonucleases.

When DNAs of various maize inbreds were digested with a number of restriction enzymes possessing four base recognition sequences, patterns similar to those in Figure 2 were seen. The length of telomeres from inbreds CM37 and T232

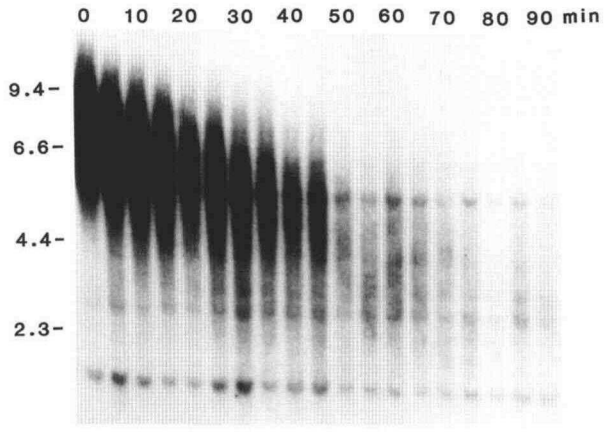

Figure 1. DNA from Inbred Tx303 Treated with BAL 31 for Varying Lengths of Time and Then Digested with DdeI.

DNA gel blots of these digests were probed with d(CCCTAAA)$_4$. The sample on the left is the 0 time control, and subsequent samples were taken at 5-min intervals. Markers at the left indicate the lengths of HindIII digested λ DNA in kilobases.

was not resolved on this gel, but the average length of the telomeres of other inbreds varied between about 15 and 2 kb. The comparatively short length of inbred WF9 telomeres is not the result of DNA degradation because the pattern of repeated sequences on the ethidium bromide-stained gel prior to blotting was similar to that of other inbreds, and the nontelomeric internal sequences homologous to the d(CCCTAAA)$_4$ oligonucleotide were the same size as those in most of the other inbreds. We have examined a total of 22 inbreds and all fall between the extremes shown in Figure 2. Their lengths are given in Table 1. Overall, there is a 25-fold variation among these strains.

Similar telomere lengths were observed when DNA was digested with 12 other restriction endonucleases having four or five base pair recognition sequences (AluI, AvaII, BstNI, FokI, HaeIII, HinfI, HphI, MnlI, RsaI, Sau3AI, Sau96I, TaqI). None of the three 5-methylcytosine-sensitive enzymes (Fnu4HI, MspI, and NciI) we used digested the subtelomeric DNA. The telomeres themselves do not appear to be cleaved by any of these enzymes. These results suggest that penultimate to the telomere repeat is a region susceptible to digestion by a number of restriction enzymes that cleave DNA frequently.

To elucidate sequences proximal to the telomeres, we developed a strategy for cloning these regions that made use of the polymerase chain reaction. We took advantage of the fact that digestion of maize DNA with many four base recognition restriction enzymes produces mostly small fragments. This allows a significant enrichment of the higher molecular length telomeric DNA on preparative agarose gels. Telomeric DNA isolated in this fashion is presumed to have a small amount of the proximal sequence. If the enzyme employed produces a staggered cut, these fragments can be ligated together in a tail to tail fashion. We used the polymerase chain reaction to amplify the region between the telomeric repeats in the self-ligated molecules joined via their proximal sequences. The telomeric repeats are presumed to be oriented such that the C-rich strand is oriented 5′ to 3′ from the end of the chromosome toward the centromere (Zakain, 1989). The d(CCCTAAA)$_4$ primer would therefore be expected to be the most efficient primer for amplifying from the self-ligated templates. It was, but the d(TTTAGGG)$_4$ primer was also able to amplify using these templates, albeit at somewhat lower efficiency. We have no evidence that maize telomeres are constituted of multiple repeats of the same heptanucleotide oriented in one direction. Only a smear of low molecular weight fragments was detected when the unligated isolated fragments were used as templates.

Two clones from the amplification of AvaII digested and self-ligated telomeres and one obtained by Sau3A digestion were isolated and sequenced. Examination of the sequences presented in Figure 3 indicates that there is a second AvaII site in the insert in pBF266 and a second Sau3A site in the insert of pBF268. It is possible that in both cases one of the two sites was not cleaved in the preparation of the fragments prior to ligation. Recognition sites of the enzymes we successfully used to reveal telomeres on DNA gel blots, with the

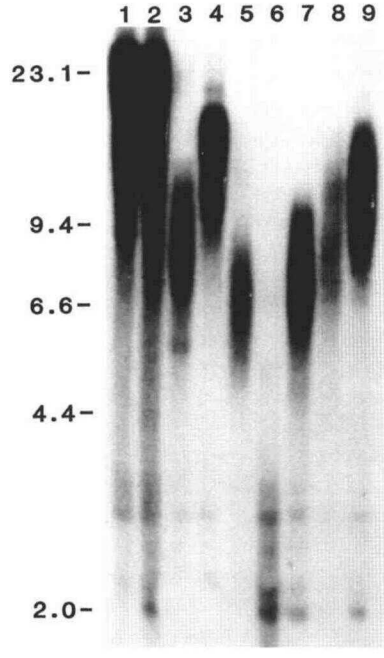

Figure 2. Polymorphism of Telomere Length in Maize Inbreds.

DNA was digested with DdeI prior to DNA gel blotting and hybridization with d(CCCTAAA)$_4$. Lane 1 contains DNA from inbred CM37; lanes 2, T232; lane 3, Tx303; lane 4, CO159; lane 5, B37; lane 6, WF9; lane 7, W22; lane 8, M14; lane 9, Oh43. Length markers at the left are in kilobases.

Table 1. Length of Telomeres in Maize Inbred Lines and Some of Their F_1 Hybrids

Inbred or F_1	Number Average Length (kb)
A671	9.2
B37	7.0
B73[a]	2.8
B73[b]	3.2
B73 × Mo17	4.3
CG16	17.4
CH593-3	8.3
CH606-11	3.3
CK52	20.0
CM37	40.0
CO159	12.9
CO159 × Tx303	8.1
CO220	4.1
De811	5.0
De811 × B73	3.6
M14	8.5
Mo17	8.8
N28	8.8
ND300	24.0
Oh43	10.6
Pa326	8.6
W22	7.1
WF9	1.8
WF9 × B37	6.1
T232	39.4
Tx303	7.7

[a] This version of B73 was provided by Agrigenetics Co.
[b] This version of B73 was provided by Pioneer Hi-Bred International.

exception of FokI and HphI, were found to be present in at least one of these sequences. A number of repeated sequences are located within the three clones and these are listed in Table 2. It should be noted that one of the 122-bp repeats spans a potential junction site in pBF268. All three clones detect two major NcoI repeats of 1.1 and 0.8 kb, suggesting that they are parts of the same degenerately repeated family. We used the slot blotting technique (Rivin et al., 1986) to estimate copy number of the subtelomeric repeats hybridizing to nontelomeric sequences in pBF268. The results, presented in Table 3, indicate the presence of 150 to 400 copies per haploid genome. Given the degenerate nature of the repeats, these are likely to be underestimates.

Looking for RFLPs with a probe that hybridizes to a few hundred copies per genome could be difficult. We mitigated this problem by using enzymes that either digested the repeats frequently (BglI, NcoI, or NheI) or rarely (BamHI, BglI, or EcoRI) so that the uninformative signals did not obscure the segregating bands. Inserts from pBF266, pBF267, and pBF268 were used to probe DNA blots to look for RFLPs. These sequences hybridize to a moderately repeated family and it was not difficult to find polymorphisms that segregated in either the Tx×CM or CO×Tx recombinant inbred families. We were able to map 13 of the 14 loci we scored. These loci are designated *TAS* (for *Telomere Associated Sequences*). With two exceptions, that may possibly be centromeric locations, those that show linkage with previously mapped markers are linked to the termini of eight of the 20 chromosome arms. These are listed in Table 4. Among these are pairs of RFLP loci on *1S*, *2L*, and *5S*. We suspect that the common probe recognizes a sequence repeated a number of times on each chromosome arm.

If telomere length is under genetic control, this property should segregate in a population derived from two polymorphic parents. We probed the DNA of the CO×Tx recombinant inbred population (Burr et al., 1988). Recombinant inbred DNAs from the seventh and eighth generations of selfing beyond the F_2 were analyzed. The values from the two generations are very similar and have a correlation coefficient of 0.95. Figure 4 shows the results for half of this population from the eighth generation. Telomere lengths do not segregate as a simply inherited trait; rather, the size classes vary continuously as is characteristic of a multigenic trait. Sizes vary between lengths shorter than parental inbred Tx303 to a few that are larger than inbred parent CO159.

In Figure 4 a weak band at 5.8 kb can be observed in some of the recombinant inbreds. This band is an internal sequence

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A          10         20         30         40         50
AACCCTAAAC CCTAAACCCT AAACCCTAAA CCCTACAGCT CGTGGGAGGC
CAAGAACAAC TTTAAGGTGC TCTGGGGACC AAAACGCGGG TGGTGGAACA
CGTACTTGAA AATGGTTGCT CGACCATGAA AATGTGTGCT ATAGCTTACG
GGAGGCCATA AACAGCCTTA GCAGTCTCAG TGGCCAAATC ATGGGTGGCG
GACGGGACGC ATGAAATCGT TGTTTGACGA TGAGAACATG TGTCACGAGA
GGCAAAAAAT AGCCTTACCG GCCTCCGGGA CCACAATGTG TACTATGGTT
TGGACACTAA AATACTACTA CGTCAGCAAA GCTGATATGG TTGGGAGACT
CAAATTTCAT TTTACCATGG TTTCGACACT AA

B          10         20         30         40         50
CCTAAACAGT AAACCCCAAA CCTGAACACC CAAAACACCA AACCCGAACT
ACAAACACCT AACATTGAAC CCCGAACACC AAAATCATAA ACCCTAGACC
CCGAGCCACG AACACTAAAC AATGAACCCC GAACCTTAAA ACTAAAACGT
GACACCCAAA ACACAAAACC TCAAACTCAA ATCCTAGACC ATATCAACTT
TGCTGACATA GTAGAATTTT AGTGTCCAAA CCATAGTACA TGTTTTGGTC
CCTGGAGGCC GGTAAGGCTA TTTTTGGCCT CCCGTGACAC ATGTTTTCAT
CGTCAAACAA C

C          10         20         30         40         50
CCTAAACCCT AAACCCTAAA CCCTAAACCC TAAACCCTAA ACCCTAAACC
CTAAACATCA TAACCTAGAC CCTATGTATC TAGTGCTTAC ACCTATGCAT
TTTTTAGCAT TAAATGATTT CCACAAAAAA CCCAAGAATG GGAATTGTGT
CCAACCAATA TTAAAATGTA ATCCAAGTAA TAGTAAAATG CAAAGATCAT
AAAAAAATTA AGTGCAAACC ATTGTAGAAT TTTAGAGATA GTACAATTTA
TCTAAACCTT GATAAATATC TAGAGACAGA AAAAATTTGC CCAAACAATA
AACAATAAAC ACTAAAACAC AAACCCTTAA CTCGAAACTC TAAACCCCAA
ACCTTAAACC CTGTATATCG AAGGATAACT CATATCGCTT TTTGACCTTT
ACGATCCCCA ACTAAGGAAC CAAGATTGTG ATTTGTGTTT TAGGGTTTAG
GGTTTAGGGT TTAGGGTAC
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Figure 3. Sequences of Self-Ligated Subtelomeric Sequences.

(A) Insert of pBF266, 382 bases.
(B) Insert of pBF267, 311 bases.
(C) Insert of pBF268, 469 bases.
The recognition sequence used to generate the telomeric fragments prior to self-ligation and amplification is underlined.

Table 2. Repeated Sequences in Subtelomeric Clones

First Occurrence	Position	Second Occurrence	Position	Length (bp)	Homology (%)
pBF266	33–77	pBF266	139–183	45	66
pBF266	219–340	pBF267	311–219	122	92
pBF268	68–120	pBF268	357–409	53	65
pBF267	100–210	pBF268	299–307	111	62

hybridizing to the d(CCCTAAA)$_4$ probe; it is polymorphic in the CO×Tx population, and its presence or absence behaves as a unitary character. The 5.8-kb band maps to the locus *BNL17.17* at position *43* in the middle of chromosome arm *8L*. A 2-kb DdeI band segregating in the Tx×CM population also maps to the same locus.

More than one gene controlling telomere length is segregating for telomere length in the CO×Tx recombinant inbred family. Multiple regression by leaps and bounds (Romero-Severson et al., 1989) was used to determine which previously mapped markers were most tightly associated with telomere length. In the most conservative model, two markers, *GLN1* on *10S* and *BNL8.23* on *4L*, accounted for 45% of the observed variation. The inclusion of a third marker, *YNH20* on *1L*, increased this to 50%. The probability of this model occurring by chance is less than 0.00001, and the individual probabilities that the three loci were obtained by chance are less than 0.001, 0.010, and 0.055, respectively. The same telomere data have also been analyzed by interval mapping followed by simultaneous mapping by Knapp et al. (1992). In their most conservative model, three regions accounted for 72% of the variation. Two of these intervals included *YNH20* and *GLN1*; a third was localized on *4S*. This is a trait with apparent high heritability and we presume that other marker loci were not detected. The CO×Tx family, with 41 members, is a relatively small population, and it is difficult to distinguish those loci that have a less significant effect from random nongenetic events. The regression model can be used to predict phenotype from genotype (Sokal and Rohlf, 1981). The effects of *GLN1*, *BNL8.23*, and *YNH20* were used to predict telomere lengths of the inbred parents Tx303 and CO159 and their F$_1$ hybrid. The results, given in Table 5, indicate that the loci linked to these two markers are excellent predictors of telomere length in this population.

Table 3. Estimation of the Copy Number of Subtelomeric Repeats

Inbred	Copy Number
CM37	400
T232	150
Tx303	380
CO159	180

Genome equivalents of sequences hybridizing to the internal 208-bp Sau3A fragment of the insert in pBF268 are given. See Methods for details.

Examination of telomere length in F$_1$ hybrids between inbreds with different lengths can tell us something about the additivity or dominance of the alleles affecting this trait. The results for hybrids between CO159 and Tx303, the parents of the recombinant inbred family in which telomere length was segregating, and between inbreds WF9 and B37 are presented in Figures 5A and 5B. F$_1$ hybrids of two additional crosses are listed in Table 1. There are two points to be made about these results: the F$_1$ hybrids have a discrete size; they are not a mixture of the parental bands. The bands are not necessarily intermediates of the parental types; in the cases of CO159 × Tx303 and WF9 × B37, they are the sizes of one or the other of the two parental bands.

DISCUSSION

We confirmed that the d(CCCTAAA)$_4$ probe was directed to telomeres because the sequences recognized were highly sensitive to digestion by the BAL 31 exonuclease. Furthermore,

Table 4. Telomere-Associated Sequences Mapped to the Ends of the Lineage Map

Locus	Map Position[a]	Distance from Nearest Marker (cM)[b]
TAS1H	*1S, −51*	13
TAS1C	*1S, −38*	11
TAS1A	*2S, −7*	4
TAS1P	*2L, 197*	6
TAS1G	*2L, 212*	15
TAS1B	*3S, −10*	17
TAS1E	*4S, 3*	6
TAS1O	*4L, 178*	5
TAS1N	*5S, −50*	3
TAS2B	*5S, −47*	8
TAS1M	*8L, 109*	17

[a] We have attempted to key the coordinates of our molecular map to the well-established maize morphological map in which position *0* is defined by the most distal marker on the short arm. Because molecular markers extend the range of the map, we have had to resort to the use of negative numbers on the distal ends of most short arms.
[b] Distance from the nearest marker mapped in the T×CM and CO×Tx recombinant inbred populations.

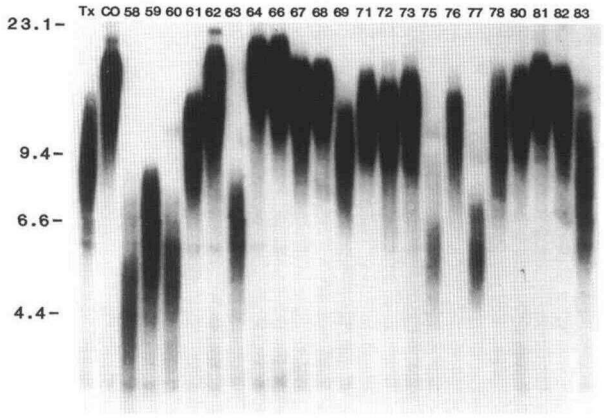

Figure 4. Segregation of Telomere Length in a Recombinant Inbred Family.

DNA of the parents and half of the CO×Tx recombinant inbred family (Burr et al., 1988) was digested with DdeI, prepared for DNA gel blotting, and probed with $d(CCCTAAA)_4$. At the top of the figure, Tx and CO refer to parental inbreds Tx303 and CO159; these are followed by the numbers of the individual recombinant inbreds. Length markers at the left are in kilobases.

probes developed from adjacent sequences detect loci (*TAS*) that map to the ends of our linkage maps.

At the DNA level, maize is the most polymorphic species that has been studied (Evola et al., 1986; Shattuck-Eidens et al., 1990). The considerable amount of variation in telomere length is undoubtedly another manifestation of the heterogeneous nature of the species. Although it is not strictly comparable—given the different mechanism of telomere synthesis—this result recalls the variability reported for copy number of repeated sequences between inbred lines of maize (Phillips, 1978; Riven et al., 1986). Despite the variability between genotypes, copy number is stable within a genotype for telomeres and repetitive sequences. We have observed no phenotypic effect of the telomeric length polymorphism. Both WF9, the line that has the shortest telomeres measured in this study, and T232, one of the lines with the longest telomeres, are robust inbreds.

We were aided in this study by having the Tx×CM and CO×Tx recombinant inbred families (Burr et al., 1988). These are permanent mapping populations that have been extensively mapped. The current data base contains more than 700 mapped markers. Mapping new, simply inherited loci is easily carried out (Burr et al., 1988). The dense map also is useful for finding linkage with genes having major effects on quantitative traits. However, the CO×Tx population, which contains 41 members, is probably too circumscribed to detect with certainty those loci having a small effect on telomere length. We plan to examine an extended set of recombinant inbreds generated from the same two parents to verify or rule out the weakly acting loci that were detected.

One hypothesis current among quantitative geneticists is that genes affecting both quantitative and qualitative traits are part of the same spectrum. The same locus may have functional alleles, expressing quantitative variation, or null alleles having Mendelian inheritance (Robertson, 1985). One can speculate about the nature of the loci we have identified. Given the special replicative process associated with telomeres, it is possible that we are observing variation in the levels of activity of genes or their products that are responsible for telomere synthesis or maintenance. Such mutations have been described in yeast (Carson and Hartwell, 1985; Lustig and Petes, 1986; Lundblad and Szostak, 1989; Lustig et al., 1990). These mutations affect all telomeres coordinately. In fact, genetically determined differences in telomere length have been observed among yeast strains with telomeres that differed by 200 to 250 bp in length (Horowitz et al., 1984; Wamsley and Petes, 1985). Diploids had intermediate lengths and meiotic progeny of these strains segregated for parental and intermediate telomere lengths, suggesting that two or more unmapped genes were segregating.

In the CO×Tx recombinant inbred population, we mapped three segregating loci with polymorphic alleles that affect telomere length. The parents must be homozygous for both positive and negative alleles at several loci because segregants in the recombinant inbred family have telomeres that are either larger or smaller than those of the parents. Because additive and dominance genetic effects appear to be the norm in maize quantitative inheritance (Sprague, 1983), we expected the F_1 progeny, being heterozygous for both positive and negative alleles at all segregating loci, to have the most favorable combination of positive alleles and therefore exhibit longer telomeres than either parent. As shown in Figures 5A and 5B, however, this is not the case. The hybrid of CO159 × Tx303 has an average telomere length approximately the size of the parent with shorter telomeres, whereas that of WF9 × B37 is the size of the parent with long telomeres. In the former case, this may result from dominant negative effects of some parental alleles. The inbred WF9 has the shortest telomeres of the lines surveyed. If it has no positive alleles with respect to the loci that are polymorphic with B37 for telomere length, the F_1 result could be due to complete dominance of the B37 alleles.

Table 5. Predicted Telomere Lengths Based on the Genotypes of *BNL8.23*, *YNH20*, and *GLN1*[a]

Genotype	Predicted	Observed
Homozygous for Tx303 parental alleles	7.8 ± 2.8	7.7 ± 0.7
Homozygous for CO159 parental alleles	11.2 ± 2.6	12.9 ± 0.6
Heterozygous for all parental alleles	9.5 ± 2.5	8.1 ± 0.6

[a] Lengths given in kilobases ± SE.

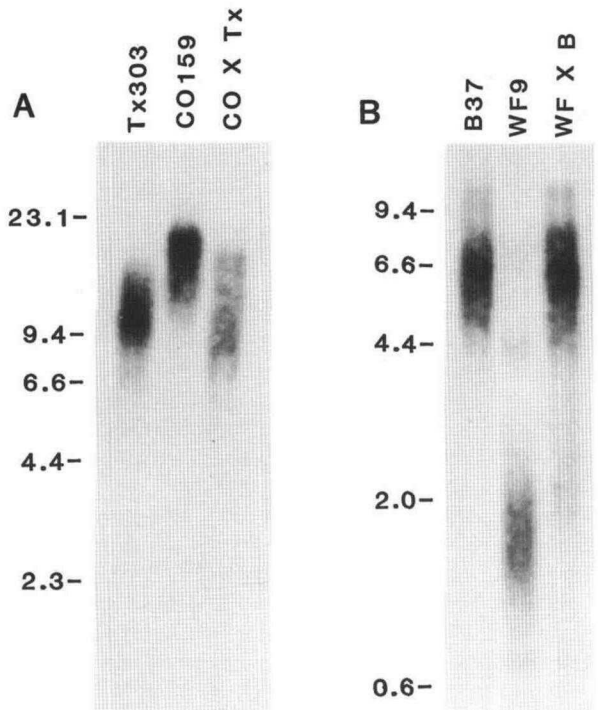

Figure 5. Comparison of F_1 Hybrid Telomeres with Inbred Parents.

(A) DNA from inbreds Tx303, CO159, and their F_1 hybrid were digested with DdeI, subjected to DNA gel blotting, and probed with d(CCCTAAA)$_4$.

(B) DNA digested with TaqI from inbreds B37, WF9, and their F_1 hybrid.

Length markers at the left are in kilobases.

Despite the considerable polymorphism, telomere length is presumed to have high heritability because the lengths observed in two generations are very similar. Moreover, samples from individuals of the same genotype exhibit identical telomere lengths. The degree to which genotype influences telomere length is evidenced by the rapidity in which telomeres of F_1 hybrids come to a distinctive length. All of the DNA samples were prepared from newly expanded leaves taken from the upper part of the plant. Assuming equal cell division, we calculate that about 47 doublings are required to achieve a mature leaf from a zygote. Otto and Walbot (1990) estimated that there are about 20 generations between the zygote to apical meristem in the mature embryo. We calculate that another 27 doublings are needed to form a mature leaf: based on a yield of 1600 μg of DNA per leaf and 10 pg per diploid cell, there are 2^{27} cells per leaf. Thus, F_1 plants probably achieve their distinctive telomere lengths in less than 50 cell generations. In contrast, 100 to 150 generations are required for yeast mutants to reach the full extent of their expression on telomere length (Carson and Hartwell, 1985; Lustig and Petes, 1986; Lundblad and Szostak, 1989; Lustig et al., 1990). Perhaps the tighter genetic control in maize can be explained by postulating the existence of actively competing forces—gene products that are responsible for telomere degradation as well as those that are involved in their synthesis.

METHODS

Plant Material

Zea mays inbred lines and F_1 hybrids are listed below. The CO×Tx and Tx×CM recombinant inbred families have been described (Burr et al., 1988). Inbreds and F_1 obtained from D. West and D. Zaitlin, Agrigenetics, Madison, WI, were A671, B73, CG16, CH593-3, CH606-11, CK52, CO220, De811, De811 × B73, N28, ND300, and Pa326. Inbreds and F_1 obtained from C. W. Stuber, North Carolina State University, Raleigh, NC, were CM37, CO159, T232, Tx303, and CO159 × Tx303. Inbreds obtained from M. Albertsen and D. Duvick, Pioneer Hi-Bred, Johnston, IA, were B37, B73, and Mo17. Inbreds obtained from O. E. Nelson and J. Kermicle, University of Wisconsin, Madison, were M14, Oh43, W22, and WF9. F_1s produced at Brookhaven National Laboratory, Upton, NY, were WF9 × B37 and B73 (Pioneer) × Mo17.

Source of Primers and Genomic DNA

The oligonucleotides used in this study are as follows: 5′-CCCTAAACCCTAAACCCTAAACCCTAAA-3′, d(CCCTAAA)$_4$; 5′-CCCGGTACCCTAAACCCTAAACCCTAAACCCTAAA-3′, Kpn-d(CCCTAAA)$_4$; and 5′-TTTAGGGATTTGGGATTTGGGATTTGGG-3′, d(TTTAGGG)$_4$. They were synthesized on a MilliGen/Biosearch 8700 DNA Synthesizer at Brookhaven by W. Crockett. Genomic DNA was prepared (Burr and Burr, 1981) from developing upper leaves. In all cases, leaves from at least three plants were pooled to maximize the possibility of detecting heterozygosity where it was present in the inbred lines.

BAL 31 Digests

BAL 31 nuclease digests were done in 20 mM Tris, 8.3, 12 mM $CaCl_2$, 200 mM NaCl, 8 units of enzyme and 50 μg DNA in 250 μL starting volume. Aliquots were withdrawn at 5-min intervals, and the reaction was terminated with EGTA as described by Allshire et al. (1989). After heating at 72°C for 10 min, the time points were digested directly with DdeI (Bethesda Research Laboratories) after the buffer and [Mg^{2+}] had been adjusted according to the manufacturer's directions.

DNA Blotting and Nucleic Acid Hybridization

Restriction enzyme digests were electrophoresed on 0.6% neutral agarose at 1 v/cm (McDonell et al., 1977) with HindIII-digested λ DNA as molecular length markers. Larger telomeres were electrophoresed at the same voltage in 0.4% neutral agarose with recirculating buffer. In the latter case, molecular length markers were uncut bacteriophages T4, λ, and T7, and BglII-digested T7 DNA. The gels were blotted onto nitrocellulose (Burr et al., 1988). The blots were hybridized (Devlin et al., 1989) with the oligonucleotide that had been 5′ end labeled with polynucleotide kinase and γ-^{32}P-ATP.

Cloning and Sequencing of Subtelomeric Fragments

Tx303 DNA was digested with AvaII or Sau3A and separated on a 0.6% low melting temperature agarose gel. The region of the gel corresponding to 9 to 11 kb was excised and the DNA was purified with Elutip-D (Schleicher and Schuell) columns. Purified DNA was allowed to self-ligate overnight at 12.5°C. Portions of the ligation reaction were added directly to a polymerase chain reaction in the presence of 0.2 μM $d(CCCTAAA)_4$, in the case of the AvaII ligation, or Kpn-$d(CCCTAAA)_4$, in the case of the Sau3A ligation. After 10 min at 94°C, the reactions were subjected to 40 rounds of amplification at 95°C for 30 sec, 51°C for 1 min, and 2 min at 72°C. The products in the range of 560 to 430 bp from the AvaII ligation were treated with the Klenow fragment of *Escherichia coli* DNA polymerase and ligated to SmaI-digested and alkaline phosphatase-treated pT7/T3-19 or pT7/T3α-18 (Bethesda Research Laboratories). The products from the Sau3A ligation and amplification were in the range of 600 to 460 bp. These were digested with KpnI and ligated into KpnI-digested and alkaline phosphatase-treated pT7/T3α-19. Both strands were sequenced by the dideoxy nucleotide chain termination method using the Sequenase kit from United States Biochemical Corporation.

Quantitation of Subtelomeric Repeats

Genomic DNA was digested to completion with NcoI, and pBF268 was digested with EcoRI and HindIII. These digests were denatured by dilution into 0.5 M NaOH with 0.125 mg/mL salmon sperm DNA used as carrier. Genomic DNA (0.3 μg) and dilutions of 3 to 300 × 10^{-6} pmol plasmid were applied per slot in a Schleicher and Schuell Slot-Blotter to a Nytran membrane. The blot was probed with an internal 208-bp Sau3A fragment of pBF268 chosen to avoid telomeric heptanucleotide repeats. The autoradiogram was electronically imaged and areas of autoradiographic intensity were measured as described below.

Measurement of Molecular Lengths

X-ray films of telomere blots of the recombinant inbreds were electronically imaged (Freeman et al., 1986), and the number of average molecular lengths was calculated (Sutherland et al., 1987).

Marker-Based Mapping of Factors Affecting Telomere Length

Telomere lengths from DNA from field-grown plants in 1987 and in 1989, which had been self-pollinated seven and eight times after the F_2 generation, were subjected to all-subset linear regression by leaps and bounds (Romero-Severson et al., 1989) to identify a model containing a minimum number of markers that would account for the maximum amount of phenotypic variation. This model was refined by backward selection (Sokal and Rohlf, 1981) to identify that subset of markers with the most predictive power. The F statistic derived from the regression analysis gives a test of the significance of the model. F tests from different models were compared to choose the best model. A *t* test of the partial regression coefficient indicates the significance of the effect of a marker locus within a model. Probabilities were calculated from the *F* and *t* statistics (Sokal and Rolf, 1981).

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