Localization of Centromere and Telomere Sequences on Maize Pachytene Chromosomes by Fluorescence in situ Hybridization

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ABSTRACT

The centromere region of eukaryotic chromosomes includes a primary constriction (centromere proper) and the pericentric heterochromatin. The DNA component of maize centromeres consists of centromere-specific CentC satellite repeats and retrotansposons, and a variety of other repetitive sequences. Fluorescence in situ hybridization (FISH) is a powerful tool for localizing repetitive sequences. Several reports presented the localizations of these centromeric sequences on metaphase chromosomes by FISH, but the precise locations of these sequences in centromeric regions could not be ascertained because of the low resolution of metaphase chromosomes. In this study, using meiotic pachytene chromosomes of two maize inbred lines, KYS and A344, as FISH targets, we demonstrated that the CentC element is present in the primary constriction but not in the pericentric heterochromatin. In addition, we observed differences in the strength of hybridization signals among the individual chromosomes in both inbred lines. The strength of signals could be classified into two types corresponding to the appearance of centromeres based on aceto-carmine staining. Hybridization of pachytene chromosomes with the telomere repeat 5'-TTTAGG-3' revealed the differences in length of telomeres between the two inbred lines. In KYS, the cluster of telomere signals was proximal to the terminal knob on 9S. This is interpreted to be an artifact resulting from high-order chromatin folding.

Key words: centromere, telomere, Zea mays, pachytene chromosome, fluorescence in situ hybridization (FISH)

Introduction

The centromere, also known as the primary constriction on chromosomes, is essential for sister chromatid cohesion, kinetochore formation, and chromosome movement during cell divisions (Allshire, 1997; Richards and Dawe, 1998; Pidoux and Allshire, 2000). The telomere is a specialized structure at the ends of eukaryotic chromosomes that plays an important role in protecting chromosome ends from exonuclease degradation, in maintaining telomere length during chromosome replication, in preventing chromosomes from end-to-end fusion and in initiation of meiotic chromosome pairing (Zakian, 1995; Scherthan, 2001). Both centromeres and telomeres are fundamental components of eukaryotic chromosomes necessary for the faithful transmission of genetic material.

The best-characterized centromere is that of budding yeast (Saccharomyces cerevisiae). The functional centromere of this unicellular eukaryote is composed of only ~125 bp of unique sequence (Clarke, 1990, 1998). In contrast, the centromeres of fission yeast (Schizosaccharomyces pombe), Drosophila melanogaster, Arabidopsis thaliana and humans are much more complex, comprising kilobases or megabases of mostly repetitive DNA sequences (Henikoff et al., 2001). For example, the 171-bp \(\alpha\) satellite element is the major functional DNA component of the centromeres of all human chromosomes. (Wervik and Willard, 1989; Ikono et al., 1998; Henning et al., 1999). The most abundant DNA element in \(A.\) thaliana centromeres is a family of 180-bp repeats (Martinez-Zapater et al., 1986; Maluszynska and Heslop-Harrison, 1991;)

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Round et al., 1997). These repeats are organized into long tandem arrays (Jackson et al., 1998) that are interrupted by various retrotransposons (Brandes et al., 1997; Copenhaver et al., 1999; Fransz et al., 2000; Haupt et al., 2001).

Two major centromeric repetitive DNA elements have been reported in species of the grass family. One of these is satellite repeats of ~150 to 180 bp (Ananiev et al., 1998; Dong et al., 1998; Cheng et al., 2002) and the other element is derived from a Ty3/gypsy class of retrotransposons (Jiang et al., 1996; Miller et al., 1998; Presting et al., 1998; Langdon et al., 2000; Cheng et al., 2002).

In maize, two centromeric elements, CentA and CentC were isolated from an out-maize addition line for chromosome 9 of maize (Ananiev et al., 1998). CentA is a portion of the Ty3/gypsy type retrotransposon element and CentC is a 156 bp tandem repeat. Recently, Zhong et al. (2002) isolated a full length centromeric retrotransposon, CRM, from maize and found that CentC and CRM interact with CENH3, a highly conserved protein that replaces histone H3 in active centromeres, for kinetochore formation.

Fluorescence in situ hybridization (FISH) is an effective tool for localizing repetitive sequences such as centromere elements on chromosomes, and in most investigations, somatic metaphase chromosomes have been the hybridization target (Aragon-Alcaide et al., 1996; Jiang et al., 1996; Ananiev et al., 1998; Dong et al., 1998; Miller et al., 1998; Sadder and Weber, 2001; Nagaki et al., 2003). However, in plants, somatic metaphase chromosomes are too condensed for karyotyping and for observing the morphology of centromeres. Using meiotic pachytene chromosomes as target, Chen et al. (2000) successfully localized a repetitive sequence, ZmCR2.6c, to the primary constrictions of maize chromosomes and detected differences in intensities of hybridization signals among the individual chromosomes of maize. ZmCR2.6c, 309 bp in length, is a portion of long terminal repeat (LTR) of a Ty3/gypsy type retrotransposon, which shares 65% homology with the sorghum centromeric sequence pSau3A9 (Jiang et al., 1996) and 90% homology with the maize CRM element (Zhong et al., 2002). However, so far no attempts have been made to compare the distribution of the tandemly repeated CentC element in maize centromeres.

In contrast to the complex structure of centromeres, the sequences of eukaryotic telomeres are simple in that they are composed of tandem arrays of only a few base pairs. For example, the sequence of the telomere repeat in a number of higher plants, including maize, is 5'-TTTAGGG-3' (Fuchs et al., 1995; Gardiner et al., 1996). Although the sequences of the repeats are conserved, the lengths of the arrays vary from species to species, ranging from 2-5 kb in A. thaliana (Richards and Ausubel, 1988) and up to 60-160 kb in tobacco (Fajkus et al., 1995), and even from one cell type to another within an individual (Kilian et al., 1995).

In this paper, we present chromosomal locations of centromeric element CentC and telomeric repeat 5'-TTTAGGG-3' in two maize inbred lines and discuss the variation in chromosomal distribution of these sequences within and between the inbred lines.

Materials and Methods

Plant material

Zea mays L. (2n=20) inbred lines KYS and A344 obtained from the National Plant Germplasm System, United States Department of Agriculture, USA, and from Prof. W. Zacheus Cande, Department of Molecular and Cell Biology, University of California, Berkeley, CA, USA, were used in this study.

DNA probes for FISH

The maize centromeric sequence CentC (Ananiev et al., 1998) was amplified by polymerase chain reaction (PCR) using maize total genomic DNA as template and oligonucleotides 5'-AACAAGAAATTGCGAGAAACCACC-3' and 5'-GTGCACCGTCACCCCATTCCGA-3' as primers. The amplified fragments were ligated to pUC18, transformed into E. coli strain XL1-blue and screened by colony hybridization using both primers as probes. Positive clones were sequenced using an ABI PRISM 377 automatic sequencer (Perkin-Elmer). A clone containing a trimer of CentC element, pZmCentC27, was used as a probe for centromeres.

Telomere sequences were amplified by PCR in the absence of template, using oligonucleotides 5'-TTAGGG-3' and 5'-CCCTAAA-3' as primers (Cox et al., 1993). The primers serve as template
in the early PCR cycles as well, resulting in a heterogeneous population of molecules consisting of telomere repeats of various lengths. Amplification was carried out in the following conditions: 10 cycles of 1 min at 94°C, 30 sec at 60°C, and 1 min at 72°C, followed by 30 cycles of 1 min at 94°C, 1 min at 60°C, and 1 min at 72°C, and one final extension step of 5 min at 72°C. Amplified fragments were purified and used as a probe for telomerices.

**Fluorescence in situ hybridization**

Maize inbred lines KYS and A344 were used for FISH. Meiotic chromosomes were prepared according to the method of Chen et al. (1998). Probes were labeled with cy3-dUTP or FITC-dUTP (Amersham Biosciences) by nick translation. The hybridization mixture consisted of 50% formamide, 2 × SSC (20 × SSC: 3 M NaCl, 0.3 M tri-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, 2 × SSC at 42°C for 10 min, and 2 × SSC at room temperature for 3 × 5 min. Chromosomes were counterstained with 4,6-diamidino-2-phenylindole (DAPI). Slides were visualized under an Olympus
IX70 fluorescence microscope with appropriate filter sets. The images were captured using a cooled CCD camera (Photometrics) and final image adjustments were done with Adobe Photoshop 5.0 software.

Results

Chromosomal localization of CentC element

Several clones containing CentC repeat were isolated, but only four were sequenced. Of these, ZmCentC27, a trimer (GenBank accession no. AY321491), was used as a probe for centromeres. Homology among the repeating units of ZmCentC27 and those reported by Ananiev et al. (1998) is 84–94% (Figure 1).

FISH of ZmCentC27 to meiotic chromosomes of KYS and A344 showed that hybridization signals were located in the primary constrictions but not in the pericentric heterochromatin of pachytene chromosomes (Figure 2). Based on chromosomal distribution of knobs and arm ratios, all chromosomes of the two inbred lines could be unambiguously identified. Interestingly, there were two distinct sites of CentC signals on chromosome 10 in both lines; one site was stronger than the other (Figure 2A, C); the stronger signal was in the primary constriction while the small signal was in the pericentric heterochromatin on the short arm (see the magnified regions in Figure 2A, B). In both lines the intensities of CentC signals could be divided into two classes: signals on chromosomes 2, 7, 9, and 10 were stronger than those on the remaining chromosomes.

The DAPI-stained chromosomes of KYS (Figure 2A) were converted to black and white images (Figure 2B). Comparison of CentC signals in Figure 2A and the morphology of centromeres in Figure 2B revealed that centromeres of chromosomes 2, 7, 9, and 10, which showed stronger CentC signals, were stained weakly by DAPI and easily identified. Centromeres of chromosomes 1, 3, 4, 5, 6, and 8, which showed weaker CentC signals, were embedded in the pericentric heterochromatin and difficult to identify.

Chromosomal localization of telomeric repeats

The PCR-generated telomere probe was hybridized to maize pachytene chromosomes of inbred lines KYS and A344 in order to find chromosomal locations of the telomeric sequences and to compare the strength of signals within and between the two inbred lines. In A344, hybridization signals were clustered at the termini of both arms of the ten pachytene bivalents (Figure 2C). In KYS, signals were found at the termini of all chromosome arms except 9S, on which telomere signals were proximal to the terminal knob (Figure 2A). No interstitial chromosome signals were observed in both inbred lines. Telomere signals were stronger in A344 compared to those in KYS.

Discussion

In this study we have shown by FISH that the satellite repeat CentC is located in the primary constrictions but not in the pericentric heterochromatin. A similar result was reported for the sequence ZmCR2.6c (Chen et al., 2000), which is a portion of a Ty3/gypsy retrotransposon, CRM (Zhong et al., 2002). Nagaki et al. (2003) analyzed the sequences of two maize bacterial artificial chromosomes (BACs) containing CentC repeats and found that they are arranged in tandem arrays that are interrupted by various retrotransposons including CRM. Thus, our FISH results are consistent with the results from sequence analysis. It is interesting to note that rather than condense into heterochromatin, the satellite repeats in functional centromeres are more diffused than
unique sequences as they appear as constrictions on chromosomes.

Dempsey (1994) recognized two types of centromeres in acetocarmine-stained pachytene chromosomes of the maize inbred line KYS. Centromeres of chromosomes 2, 7, 9, and 10 are large, rectangular in shape, and easily identified, whereas those of the remaining chromosomes are small and circular in shape. Chen et al. (2000) found that FISH signals probed with ZmCR2.6c were weaker, more diffused, and located in the center of the first type of centromeres of Dempsey (1994), whereas signals on the second type of centromeres were stronger and more condensed. In this study we have shown that in both inbred lines, the distribution of CentC signals is opposite to that of ZmCR2.6c, i.e., signals on the centromeres of chromosomes 2, 7, 9, and 10 were stronger than signals on the other chromosomes. These results suggest that the proportion of the two centromeric elements may be different among the individual chromosome within an inbred line, but similar between the corresponding chromosomes of different lines.

The argument mentioned above is supported by the observation of two CentC hybridization sites on chromosome 10 in both inbred lines, a major site on the primary constriction and a minor site closely adjacent to the major site. It is also consistent with the result from the traditional analysis of McClintock (1978), who observed that the basic features of pachytene chromosomes in maize, except for the distribution of knob were relatively constant throughout the species. Previously, we did not detect ZmCR2.6c signals at the minor site (Chen et al., 2000). However, this does not exclude the possibility of the presence of ZmCR2.6c sequence at this position, as ZmCR2.6c is only a small portion of CRM (309 bp) so that the signals generated by it may not be detectable. If CentC and CRM interact with CENH3 protein for kinetochore formation and chromosome movement, as has been suggested by Zhong et al. (2002), then the occurrence of two CentC sites suggests that chromosome 10 is dicentric. The normal behaviour of chromosome 10 during mitosis and meiosis may be explained by the fact that the two centromeres are closely adjacent and one of them is functionally dominant due to the presence of more copies of centromeric elements.

The existence of two CentC sites on chromosome 10 may be the result of a small inversion with one breakpoint in the centromere and the other in the pericentric heterochromatin. The occurrence of two sites in the two inbred lines is interesting from an evolutionary point of view, because it may be used as a cytological marker to trace the origin of cultivated maize.

Since the knob on 9S in KYS appears to be constant from generation to generation and there is no evidence for telomere function of the repetitive sequences in knobs, the observation that telomere signals were proximal to the terminal knob on 9S is probably an artifact, caused by the high order folding of repetitive sequences in the knob. However, further investigation is needed to unravel the fact. The telomere signals in A344 were much stronger than those in KYS, suggesting that the lengths of telomeres in A344 are longer than those in KYS. Burr et al. (1992) found that the lengths of telomeres varied from 1.8 kb to 40 kb in 22 maize inbred lines.

The results of this study demonstrate that pachytene FISH is a useful technique for investigating the grass structures of centromeres and telomeres of eukaryotic chromosomes. The successful localization of centromeres and telomeres on maize pachytene chromosomes by this technique proves the way for cytogenetic mapping in this important crop.

References


The Centromere and Telomere of Maize


利用螢光原位雜交定位玉米中節與端粒序列於粗絲期染色體

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摘要

真核生物染色體的中節(centromere)區域包括一級收縮區(primary constriction)與環境中節的異染色質(pericentric heterochromatin)。目前已有許多人研究玉米中節的 DNA 是由中節特有的 CentC 重複性衛星 DNA (satellite DNA)和逆轉移子(retrotransposon)及其他多種重複性序列所組成。但有許多報告利用螢光原位雜交技術(flowroescence in situ hybridization, FISH)將這些中節序列定位在細胞有絲分裂中期(metaphase)染色體上，但由於這個時期染色體收縮得很短，測光訊號的解析力低，故不易觀察這些 DNA 序列的精確位置。在本研究中，利用收縮程度小的成數分裂粗絲期染色體(pachytene chromosome)作為螢光原位雜交標的，將 CentC 重複序列定位於 KYS 及 A3-44 兩種玉米自交系的一級收縮區上，且染色體在十個粗絲期染色體中節的強度有所不同。由 CentC 訊號強度的差異可將中節分為兩類，並發現與利用傳統 aceto-carmine 染色法所觀察到的兩種中節相互吻合。此外，以端粒序列(5'-TTAGGG-3')n 為探針，與兩種玉米自交系粗絲期染色體做原位雜交，由染色體末端所觀察到的訊號強弱差異，顯示端粒重複序列在兩種自交系中長度不同。另在 KYS 自交系中，第九條染色體短臂的端粒訊號位於末端異染色質節(knob)的內側，並非染色體的頂端。這可能是由於染色質(chromatin)高度纏繞(high-order folding)所造成的结果。

關鍵字：中節，端粒，玉米，粗絲期染色體，螢光原位雜交

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