High-Resolution Pachytene Chromosome Mapping of Bacterial Artificial Chromosomes Anchored by Genetic Markers Reveals the Centromere Location and the Distribution of Genetic Recombination Along Chromosome 10 of Rice

Zhukuan Cheng,* Gernot G. Presting,† C. Robin Buell,‡ Rod A. Wing† and Jiming Jiang*

**Department of Horticulture, University of Wisconsin, Madison, Wisconsin 53706, †Clemson University Genomics Institute, Clemson, South Carolina 29634 and ‡The Institute for Genomic Research, Rockville, Maryland 20850*

Manuscript received October 12, 2000
Accepted for publication January 12, 2001

ABSTRACT

Large-scale physical mapping has been a major challenge for plant geneticists due to the lack of techniques that are widely affordable and can be applied to different species. Here we present a physical map of rice chromosome 10 developed by fluorescence *in situ* hybridization (FISH) mapping of bacterial artificial chromosome (BAC) clones on meiotic pachytene chromosomes. This physical map is fully integrated with a genetic linkage map of rice chromosome 10 because each BAC clone is anchored by a genetically mapped restriction fragment length polymorphism marker. The pachytene chromosome-based FISH mapping shows a superior resolving power compared to the somatic metaphase chromosome-based methods. The telomere-centromere orientation of DNA clones separated by 40 kb can be resolved on early pachytene chromosomes. Genetic recombination is generally evenly distributed along rice chromosome 10. However, the highly heterochromatic short arm shows a lower recombination frequency than the largely euchromatic long arm. Suppression of recombination was found in the centromeric region, but the affected region is far smaller than those reported in wheat and barley. Our FISH mapping effort also revealed the precise genetic position of the centromere on chromosome 10.

DEVELOPMENT of molecular marker technology revolutionized genetic linkage mapping. Restriction fragment length polymorphism (RFLP) marker-based genetic linkage maps have been constructed in numerous plant species. In contrast to genetic mapping, much less effort has been devoted to physical mapping during the last decade partly due to the lack of techniques that are affordable to many labs and can be applied to different species. Thus, the majority of the genetic linkage maps developed in plant species are not integrated with any type of physical map.

Currently, three popular methods for physical mapping are used in plant species. First, DNA contigs can be assembled using large insert DNA clones, such as yeast artificial chromosomes (YACs) and bacterial artificial chromosomes (BACs). BAC contigs covering the entire genome of *Arabidopsis thaliana* have been developed (Mozo *et al.* 1999). Physical maps generated through contig assembly have the ultimate resolution. However, contig assembly is dependent on a saturated sequence-tagged site map and/or high-quality large insert DNA libraries. With the currently available technology it is expensive to develop a whole genome physical map by BAC contig assembly for plant species with very large genomes. In addition, in polyploid species or species with extensive sequence duplications it will be technically difficult to correctly assign BAC clones derived from duplicated regions to specific contigs.

A second method to generate a physical map is to locate genetically mapped DNA markers to specific chromosomal segments using cytogenetic stocks. Different types of cytogenetic stocks, including B-A translocations and oat-maize translocations in maize (Weber and Helentjaris 1989; Riera-Lizarazu *et al.* 2000), deletion stocks in wheat (Werner *et al.* 1992; Gill *et al.* 1993), and chromosomal translocation stocks in barley (Künzel *et al.* 2000) have been used in physical mapping. However, isolation of a large number of cytogenetic stocks is a daunting task. In addition, cytogenetic stocks cannot be developed in all plant species. For example, deletion stocks are generally not viable in diploid species. Additionally, the resolution of a physical map that is based on cytogenetic stocks is not only dependent on the number of stocks, but also on the accuracy of their cytological characterization. A cytologically defined chromosomal fragment can include several megabases of DNA, which could significantly limit the resolving power of the physical maps.

Corresponding author: Jiming Jiang, Department of Horticulture, University of Wisconsin, 1575 Linden Dr., Madison, WI 53706.
E-mail: jjiang1@facstaff.wisc.edu

A third method for generation of a physical map is to directly visualize DNA sequences on chromosomes by *in situ* hybridization. Application of this method has been hindered by the low sensitivity of the technique in plant chromosomal preparations. It has been technically difficult to detect small probes containing only a few kilobases of DNA using *in situ* hybridization (reviewed by Jiang and Gill 1994). Thus, the majority of the RFLP probes used in genetic linkage mapping are not suitable for *in situ* hybridization analysis. This technical difficulty can be overcome by using large insert DNA clones as hybridization probes. The repetitive DNA sequences within large insert DNA clones may cause technical difficulties for fluorescence *in situ* hybridization (FISH) analysis. Nevertheless, at least in some plant species the majority of randomly selected or RFLP marker-anchored BAC clones can be used for FISH mapping (Jiang *et al.* 1995; Zwick *et al.* 1998; Dong *et al.* 2000). Somatic metaphase chromosome-based *in situ* hybridization mapping has a relatively poor resolution because the telomere-centromere orientation of two probes that are separated by several megabases may not be resolved. Meiotic pachytene chromosomes, which are often more than 10 times longer than somatic metaphase chromosomes, have recently been used as a target for FISH mapping (Xu and Earle 1996; Fransz *et al.* 1998, 2000; Peterson *et al.* 1999; Zhong *et al.* 1999; Chen *et al.* 2000; Song *et al.* 2000). The high resolution of the pachytene FISH method, together with the recently developed fiber-FISH techniques (Fransz *et al.* 1996; Jackson *et al.* 1998, 2000), add new tools to the arsenal for fine physical mapping.

Rice (*Oryza sativa*) has become a model for monocot plants in molecular biological research (Goff 1999). The 430-Mb genome of rice is one of the smallest of monocots (Arumuganathan and Earle 1991). The RFLP-based genetic linkage maps of rice (Causse *et al.* 1994; Harushima *et al.* 1998) are among the most saturated maps in plants. A YAC-based physical map of rice covers more than half of the rice genome (Kurata *et al.* 1997). Rice BAC libraries based on *O. sativa* spp. *japonica* rice variety Nipponbare contain 92,000 clones, which cover 25 haploid equivalents of the rice genome (R. A. Wing, unpublished results). Fingerprints of 64,638 clones in the Nipponbare BAC libraries have been assembled into contigs and the BAC ends have been sequenced (Mao *et al.* 2000). Although tremendous resources have been developed for genome research in rice, the physical maps of rice are far from complete. Here we report a physical map of rice chromosome 10 based on FISH mapping of RFLP marker-anchored BAC clones on meiotic pachytene chromosomes. This map is fully integrated with the genetic linkage map. Using this physical mapping approach we discovered the correct centromere position and the distribution of recombination along the entire length of chromosome 10.

MATERIALS AND METHODS

Materials: An *O. sativa* spp. *japonica* rice variety Nipponbare and an *O. sativa* spp. *indica* rice variety Zhongxian 3037 were used for mapping BAC clones on rice chromosomes and DNA fibers. All BAC clones used for FISH mapping were identified by screening a Nipponbare BAC library (http://www.genome.clemson.edu/orders/Product.html) using RFLP markers previously mapped to chromosome 10 (Harushima *et al.* 1998). Several other DNA probes were used in the FISH analyses, including the probe pOs48 that contains a 355-bp subtelomeric tandem repeat cloned from rice (Wu and Wu 1987), the telomeric DNA probe pAtT4 cloned from *A. thaliana* (Richards and Ausubel 1988), a repetitive DNA probe pRCS2 that is specific to rice centromeres (Dong *et al.* 1998), and a rDNA probe pTa71 containing the coding sequences for the 18S•26S rRNA genes from wheat (Gerlach and Bedbrook 1979).

Fluorescence *in situ* hybridization: Young panicles of the two rice varieties containing anthers at various stages of meiosis were harvested and fixed in 100% ethanol:glacial acetic acid (3:1) Carnoy's solution. Microsporocytes at appropriate stages of prophase I were squashed in Carnoy's solution. The FISH procedure applied to meiotic chromosomes was essentially the same as that used for mitotic metaphase chromosomes (Jiang *et al.* 1995). C_ot-1 fraction of rice genomic DNA was prepared according to Jiang *et al.* (1996) and applied in the hybridization mixtures of some BAC probes. High-quality pachytene chromosome preparations were used for repeated probing. After the first round of probing and image taking the slides were soaked in 1× PBS (phosphate-buffered saline) solution to remove the coverslips. The slides were then dehydrated in an ethanol series (70%, 90%, and 100%, 5 min each), denatured again in 70% formamide at 80° for 2 min, dehydrated in a second ethanol series, and incubated with a different FISH probe(s). This procedure was repeated up to five rounds. No more than two probes, which were detected by green and red colors, respectively, were used in each round to unambiguously identify signals from specific probes. Biotin-labeled and digoxigenin-labeled probes were detected by a fluorescein isothiocyanate-conjugated antibiotin antibody (Vector Laboratories, Burlingame, CA) and a rhodamine-conjugated antidigoxigenin antibody (Roche Diagnostics, Indianapolis), respectively. Chromosomes were counterstained with 4′,6-diamidino-2-phenylindole (DAPI) in an antifade solution (Vector Laboratories). DNA fiber-based FISH procedures were according to the published protocols (Fransz *et al.* 1996; Jackson *et al.* 1998). Slides were examined under an Olympus BX60 fluorescence microscope. Chromosome and FISH signal images were captured using a SenSys charge-coupled device camera (Photometrics, Tucson, AZ) and final image adjustments were done with Adobe Photoshop 5.1 software.

Physical map construction based on locations of BACs on pachytene chromosomes: The length of the rice chromosome 10 linkage map is 83.7 cM (Harushima *et al.* 1998). For a simple comparison between genetic and physical positions of the genetic markers, the length of pachytene chromosome 10 was divided into 83.7 fractional lengths (FL). The location of each marker-anchored BAC clone on a pachytene chromosome 10 was calculated as an FL value,

$$\mathrm{FL} = 83.7\ (S \div T),$$

where S is the distance (in micrometers) from the FISH hybridization site to the end of the short arm of the chromosome, and T is the total length of the chromosome in micrometers. FL data were collected only from late pachytene chromosomes to minimize variation caused by a different degree of condensation of the two arms at early pachytene stage. At least eight

data points were collected for each BAC clone. Measurements were made on digital images using IPLab spectrum software.

RESULTS

The resolution of FISH mapping on early meiotic prophase I chromosomes: Our first goal was to examine the resolution of FISH mapping on early prophase I chromosomes of rice. The short arm of rice 10 is generally darkly stained by acetocarmine and is one of the most heterochromatic chromosomal arms in the rice genome (Cheng and Gu 1994). The different degree of chromatin condensation between the short and long arms of chromosome 10 might result in different FISH resolutions in the two chromosome arms. Therefore, two pairs of BAC probes, one pair on each arm, were used to examine the resolving power of FISH mapping on prophase I chromosomes.

The BAC clones, 45D16 and 15O22, are anchored by RFLP markers S10620 and S14152, respectively, and both have been mapped to 1.2 cM on the genetic linkage map (Harushima *et al.* 1998). These two BACs are separated by ~137 kb based on fiber-FISH analysis (Z. Cheng and J. Jiang, unpublished results). Physically, both 45D16 and 15O22 are located at the distal end on the short arm of chromosome 10 (Figure 1, C and G). The FISH signals derived from these two BAC clones were separated from each other on leptotene chromosomes (Figure 1A), but tend to be partially overlapped on zygotene (Figure 1B), early pachytene (Figure 1C), and late pachytene chromosomes (Figure 1G). The FISH signal derived from 45D16 is always toward the telomere and the telomere-centromere orientation of these two clones was consistent on both early and late pachytene chromosomes, indicating that marker S10620 is physically located distal to S14152 although they were mapped to the same genetic position.

The BAC clones 82M15 and E14I11 were identified by RFLP markers C809 and C797, which were mapped at 72.3 and 72.6 cM, respectively (Harushima *et al.* 1998). BAC fingerprinting data showed that 82M15 and E14I11 are separated by an estimated 134 kb (G. G. Presting and R. A. Wing, unpublished results). Physically these two BACs are located at the subterminal end on the long arm of chromosome 10 (Figure 1G). The FISH signals derived from 82M15 and E14I11 are well separated from leptotene to early pachytene stages (Figure 1, D–F) and become partially overlapped on late pachytene chromosomes with an unambiguous orientation in which BAC E14I11 was located toward the telomere (Figure 1G). When the two pairs of BACs were mapped on the same late pachytene chromosomes, the telomere-centromere orientations of both pairs could be resolved (Figure 1G).

The physical coverage of the genetic linkage map of chromosome 10: One of the most important criteria to judge the quality of a genetic linkage map is its physical coverage of the corresponding chromosome. The RFLP marker L769 was mapped to 0.0 cM, the north end of rice linkage group 10 (Harushima *et al.* 1998). The BAC clone 46L02 was identified by L769. The FISH signals from 46L02 were consistently proximal to the signals derived from a telomeric DNA probe pAtT4 on early pachytene chromosome 10 (Figure 1H). However, on late pachytene chromosomes the signals from these two DNA probes overlap completely and their orientation could not be resolved (Figure 1I). The physical distance between 46L02 and pAtT4 is likely shorter than that between 45D16 and 15O22 based on the resolution of the signal orientation on both early and late pachytene chromosomes. Thus BAC 46L02 is likely <137 kb away from the distal end of the short arm of chromosome 10.

The RFLP marker C405 was mapped to 83.7 cM, the south end of rice linkage group 10 (Harushima *et al.* 1998). The BAC clone 56G17 was identified by C405. The FISH signals derived from 56G17 and pAtT4 overlap completely on chromosomes from zygotene (Figure 1J) to late pachytene stages (Figure 1K). On the basis of the FISH signal patterns on chromosomes at similar meiotic stages it is evident that the physical distance between 56G17 and pAtT4 is shorter than that between 82M15 and E14I11.

The distal ends of chromosome 10 in *indica* rice contain repetitive sequences that are missing in the corresponding regions of chromosome 10 in *japonica* rice (Figure 2A). The distal end of the short arm of chromosome 10 in *indica* rice is marked by a 17S-5.8S-25S ribosomal RNA gene locus (Fukui *et al.* 1994; Figure 2A). When BAC 46L02 was cohybridized with the rDNA probe pTa71 on *indica* rice chromosomes, the signals derived from the two probes partially overlapped (Figure 2B). Fiber-FISH analysis was conducted to examine the physical distance from 46L02 to the rDNA sequences. The size of the gap separating the signals from 46L02 to pTa71 is ~58% of the insert of BAC 46L02 (144 kb). This gap was estimated as 84 ± 5 kb on the basis of five measurements (Figure 2C). It is not known if the telomere is directly associated with the rDNA locus on chromosome 10 in *indica* rice.

The distal end of the long arm of chromosome 10 in *indica* rice is marked by a locus of the Os48 repeat family (Figure 2A). Os48 is a tandemly repeated sequence and is organized as long arrays of a 355-bp monomer (Wu and Wu 1987; Cheng *et al.* 2001). The FISH signals derived from 56G17 and pOs48 can be resolved on early pachytene chromosomes with signals from pOs48 located distally to those of 56G17 (Figure 1D). Fiber-FISH analysis in *indica* rice cultivar Zhongxian 3037 showed that the gap separating the signals from 56G17 to pOs48 is ~30% of the insert size of BAC 56G17 (131 kb). The gap was estimated as 40 ± 2 kb from five measurements (Figure 1E). The Os48 repeats are tightly associated with the telomeric sequences (Wu *et al.* 1994;

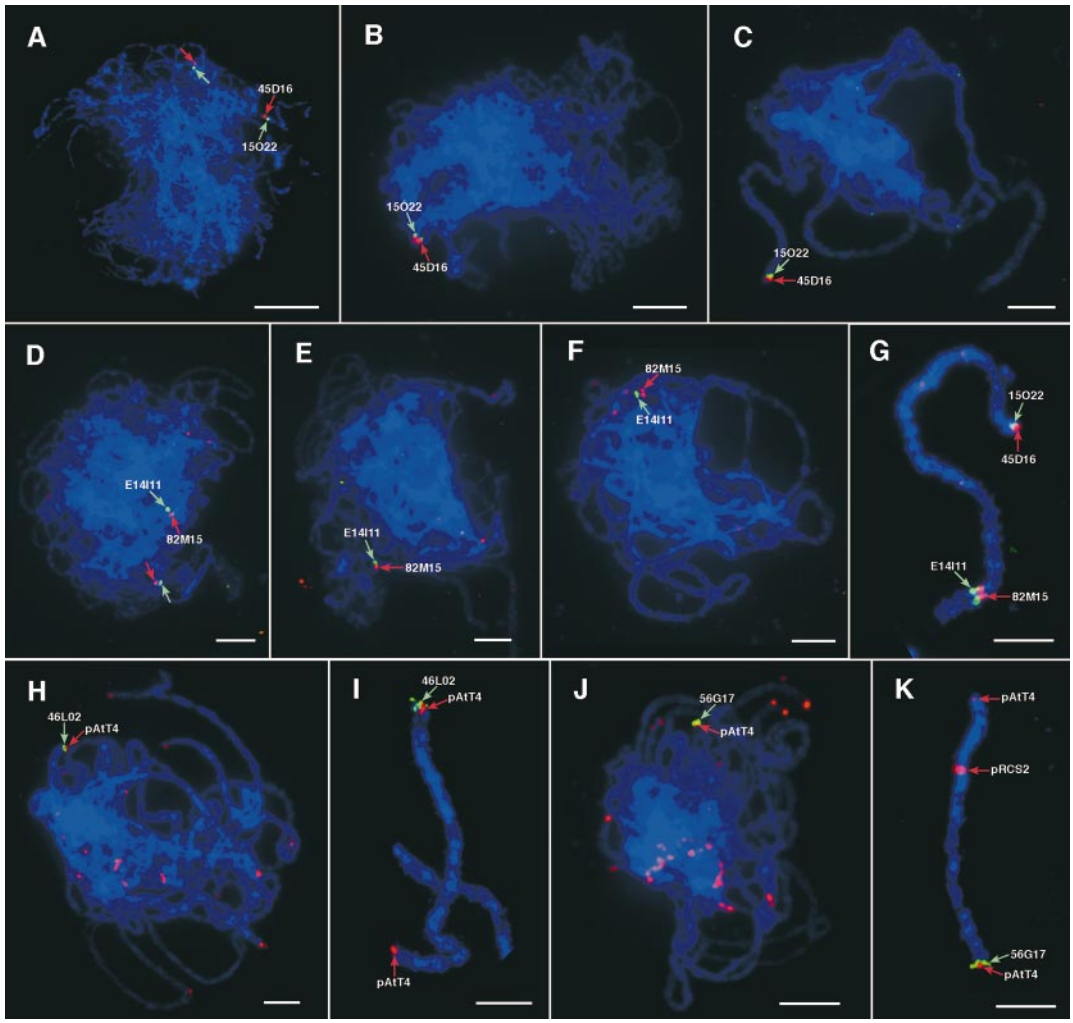

FIGURE 1.—Resolution of rice pachytene FISH mapping and the physical coverage of the rice genetic linkage map 10. (A) Leptotene. (B) Zygotene. (C) Early pachytene chromosomes hybridized to BAC clones 45D16 (red) and 15O22 (green), which are separated by ~137 kb. (D) Leptotene. (E) Zygotene. (F) Early pachytene chromosomes hybridized to BAC clones 82M15 (red) and E14I11 (green), which are separated by ~134 kb. (G) A late pachytene chromosome 10 hybridized to BACs 45D16 (red), 15O22 (green), 82M15 (red), and E14I11 (green). (H) Early pachytene chromosomes were hybridized to BAC 46L02 (green) and a telomeric DNA probe pAtT4 (red). (I) A late pachytene chromosome 10 hybridized to 46L02 (green) and pAtT4 (red). (J) Late zygotene chromosomes were hybridized to BAC 56G17 (green) and pAtT4 (red). (K) A late pachytene chromosome 10 hybridized to 56G17 (green), pAtT4 (red), and pRCS2 (red). All chromosomes were prepared from *japonica* rice Nipponbare. Bars, 5 μm.

CHENG *et al.* 2001). However, the physical distance between the Os48 locus and the telomere on chromosome 10 is not known.

The genetic position of the centromere of chromosome 10: The centromere position on chromosome 10 had been previously mapped to between RFLP markers S17868 and C1166 at 24.3 and 29.8 cM, respectively (HARUSHIMA *et al.* 1998). This position was deduced by dosage analysis of genetically mapped RFLP markers using a chromosome 10 short-arm telotrisomic stock (2n + •10S; SINGH *et al.* 1996; HARUSHIMA *et al.* 1998).

The RCS2 repeat is highly specific to the centromeric regions of rice chromosomes (DONG *et al.* 1998). Cross-hybridization of probe pRCS2 to regions other than centromeres has never been detected on rice pachytene chromosomes. Using the FISH signals from pRCS2 as a reference, the arm locations of BAC clones anchored by genetically mapped RFLP markers can be unambiguously determined. BAC E30A10, identified by RFLP marker G1125 at 15.4 cM, was mapped to the short arm of chromosome 10, while BAC 53D03, isolated by marker C489 at 15.9 cM, was localized on the long arm of chromosome 10 (Figure 3A). All BACs on the north side of 15.4 cM were mapped to the short arm, while all BACs on the south side of 15.9 cM were mapped to the long arm (Figure 3, B and C). These results demonstrate that the centromere of chromosome 10 is located between markers G1125 (15.4 cM) and C489 (15.9 cM), respectively.

The genetic and physical distances between RFLP

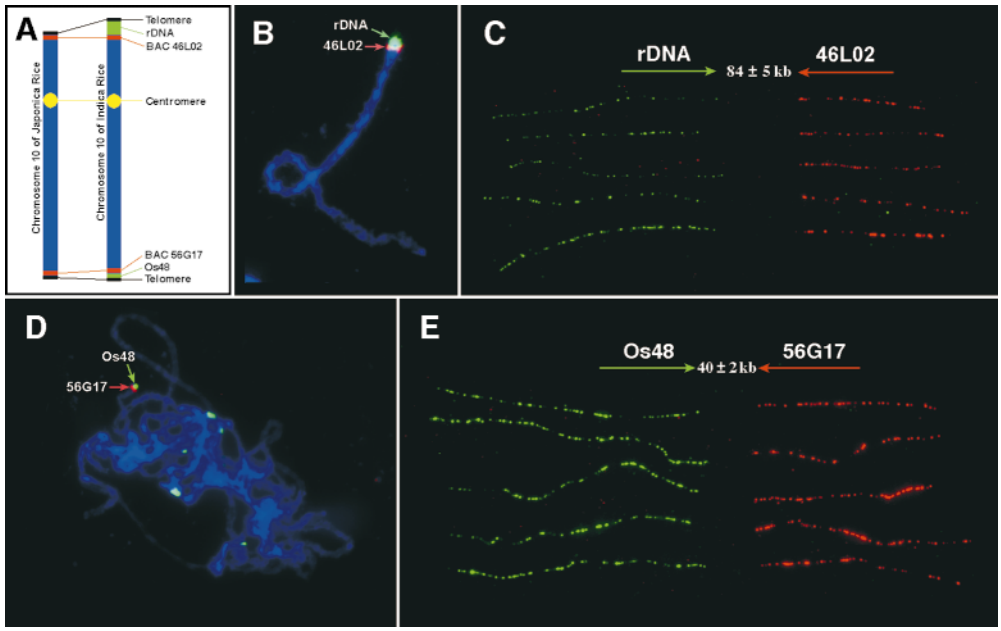

FIGURE 2.—Physical mapping of the most distal BAC clones 46L02 and 56G17 in an *indica* rice variety Zhongxian 3037. (A) Diagrams of chromosome 10 from *japonica* and *indica* rice. The rDNA locus and an Os48 locus are located at the distal ends of the chromosome 10 of *indica* rice. These two loci are not found on chromosome 10 of *japonica* rice. The relative sizes of the colored bars do not correspond to the physical sizes of the DNA loci. (B) A pachytene chromosome 10 of Zhongxian 3037 hybridized to BAC 46L02 (red) and pTa71 (green, the nucleolus organizer region). (C) Fiber-FISH images from hybridization of DNA fibers of Zhongxian 3037 to probes 46L02 (red) and pTa71 (green). Only part of the signal (toward the gap) derived from pTa71 is shown in the images, whereas the signals from 46L02 are complete. (D) Pachytene chromosomes of Zhongxian 3037 hybridized to BAC 56G17 (red) and pOs48 (green). (E) Fiber-FISH images from hybridization of DNA fibers of Zhongxian 3037 to probes 46L02 (red) and pOs48 (green). Only part of the signal (toward the gap) derived from pOs48 is shown in the images, whereas the signals from 56G17 are complete.

markers mapped on chromosome 10: To investigate the relationship between genetic and physical distances along the entire length of chromosome 10, we mapped the pachytene chromosome positions of 18 BACs anchored with RFLP markers (Figure 3, B–D). The 18 RFLP markers are separated by an average of 4.7 cM. The mapping results are summarized in Table 1 and Figure 4.

In general, recombination is evenly distributed along the physical length of chromosome 10 (Figure 4). The short arm, however, shows a lower recombination frequency (15.4 cM/20.7 FL) than the long arm (67.8 cM/59.5 FL). A significant disproportion between genetic and physical distances was found in the centromeric region and a second region that spans 58.4–71.2 cM. G1125 (15.4 cM) and C489 (15.9 cM) are the closest RFLP markers flanking the centromere. These two markers are separated genetically by only 0.5 cM but physically by 3.5 FL, a 7-fold difference. Another pair of centromeric flanking markers, R2174 (13.5 cM) and C148 (17.5 cM), are separated genetically by 4 cM but physically by 14.3 FL, a 3.6-fold difference.

The current genetic map of chromosome 10 contains two large linkage gaps that span 59.6–68.4 and 72.6–82.9 cM, respectively. To reveal the physical nature of these two linkage gaps we isolated BAC clones that closely flank these gaps. BAC E18B10, anchored by C488 at 58.4 cM, and BAC E15I11, anchored by C16 at 71.2 cM, flank the first gap and are separated genetically by 12.8 cM. Physically these two BACs are separated by only 2.7 FL (Figure 3D), indicating that this region has a higher recombination rate than the rest of the chromosome. BAC E14I11, anchored by C797 at 72.6 cM, and BAC 3O19, anchored by C239 at 82.9 cM, flank the second gap. These two BACs are genetically separated by 10.3 cM and physically by 7.5 FL (Figure 1D), suggesting an average ratio between genetic and physical distances within this region. Since these two gaps are located close to each other, the difference of recombination frequency associated with these two linkage gaps can be clearly demonstrated by pachytene chromosome FISH (Figure 1D).

DISCUSSION

The resolving power of pachytene FISH: The high resolution of pachytene chromosome-based and DNA fiber-based FISH methods has significantly increased the power of DNA *in situ* hybridization as a fine physical mapping tool. A major advantage of pachytene FISH over fiber-FISH mapping is that the centromere-telomere orientation of the FISH probes can be visualized. The resolving power of pachytene FISH depends on three factors. First, the degree of chromatin condensation at the pachytene stage may vary significantly among different plant species. Thus, pachytene FISH may have different resolving power in different plant species. Second, the location of probes in euchromatic or heterochromatic regions will affect the FISH resolution. In tomato, pachytene FISH can resolve probes separated by 1.2 Mb in heterochromatic regions and 120 kb in euchromatic regions (De Jong *et al.* 1999). However,

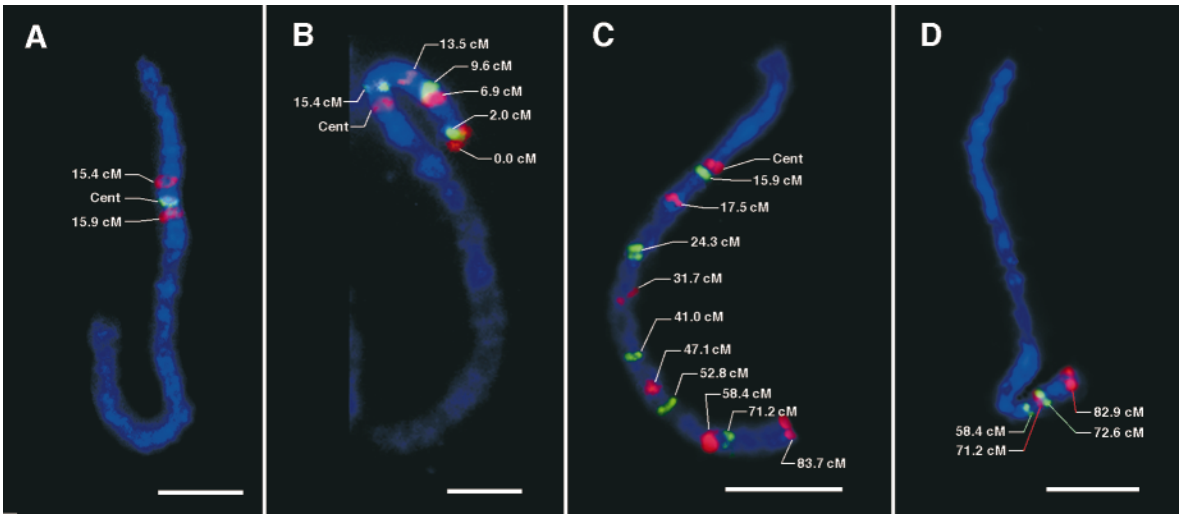

FIGURE 3.—FISH mapping of RFLP marker-anchored BAC clones on rice pachytene chromosome 10. (A) The genetic position of the centromere is mapped between marker G1125 (BAC E30A10, 15.4 cM) and marker C489 (BAC 53D03, 15.9 cM). Bar, 10 μm. (B) FISH mapping of 6 short-arm BAC clones and the centromeric DNA probe pRCS2 on the same pachytene chromosome. Bar, 5 μm. (C) FISH mapping of 10 long-arm BAC clones and the centromeric DNA probe pRCS2 on the same pachytene chromosome. Bar, 10 μm. (D) FISH mapping of 4 BAC clones flanking the two linkage gaps (58.4–71.2 and 72.6–82.9 cM) in the distal region on the long arm. Bar, 5 μm. All images were obtained by repeated probing on the same pachytene chromosomes.

the resolution in euchromatic and heterochromatic regions in *A. thaliana* are 60 and 140 kb, respectively, because the chromatin of *A. thaliana* is much less condensed than those of tomato at the pachytene stage (DE JONG *et al.* 1999). Third, the resolving power also depends on the different pachytene substages. The sizes of late pachytene chromosomes of rice range from ~25 μm (the shortest chromosome) to ~60 μm (the longest chromosome). However, early pachytene chromosomes are significantly longer than chromosomes in later stages. In the present study, probes pAtT4 and 46L02 (Figure 1, H and I) as well as probes pOs48 and 56G17, which are separated by 40 kb (Figure 2D), can be resolved on early but not late pachytene chromosomes. Because the resolving power of pachytene FISH is affected by these three factors, great caution should be paid to convert the microscopic distance, which separates the two DNA probes on pachytene chromosomes, into kilobases.

Genetic position of the centromere of rice chromosome 10: The centromere positions in the rice genetic linkage maps developed by HARUSHIMA *et al.* (1998) were deduced by dosage analysis of RFLP markers using a series of secondary trisomics (2n + one isochromosome) and telotrisomics involving every rice chromosome, a strategy first demonstrated by SINGH *et al.* (1996). A chromosome 10 short-arm telotrisomic stock (2n + •10S) was used to locate the centromere of chromosome 10 (SINGH *et al.* 1996; HARUSHIMA *et al.* 1998). In this study, we demonstrate that the centromere of chromosome 10 is located between map positions 15.4 and 15.9 cM, rather than between 24.3 and 29.8 cM determined by telotrisomic analysis (HARUSHIMA *et al.* 1998). It is likely that the extra chromosome in the 10S telotrisomic stock used in centromere mapping by SINGH *et al.* (1996) is not a true telocentric chromosome, but an acrocentric chromosome containing the short arm, the centromere, and a small proximal part of the long arm. This hypothesis is supported by two recent research experiments. First, the genetic positions of rice centromeres have been determined by linkage mapping of centromere-specific repetitive DNA elements (WANG *et al.* 2000). The map positions of all the rice centromeres, except chromosome 10, determined by this different approach were located within the linkage blocks containing the centromeres deduced from DNA dosage analysis using telotrisomics and secondary trisomics. The centromere position of chromosome 10, however, is located on the north side of the centromere-containing linkage block (WANG *et al.* 2000). Second, DNA dosage analysis was conducted to locate the centromere of chromosome 10 using an isotetrasomic stock of 10S (2n + 10S•10S + 10S•10S; CHENG *et al.* 1997). The results suggested that the centromere is located between marker G1125 (15.4 cM) and G1084 (18.6 cM), in agreement with the present study.

Relationship between genetic and physical distance: Significant discrepancies between genetic and physical distances have been reported in a number of plant species. In wheat and barley, both with relatively large genomes and large-sized chromosomes, recombination is mainly distributed along the distal half of the chromosomes. Recombination in the centromeric regions, which may account for as much as 50% of the length of each chromosome, is essentially suppressed (WERNER *et al.* 1992; GILL *et al.* 1993; DELANEY *et al.* 1995a,b;

TABLE 1

Genetic and physical locations of RFLP markers and their corresponding BAC clones

BAC clone	RFLP marker	cM	FL	No. of measurements
46L02	L769	0.0	0.00 ± 0.00	8
45O16	S10620	2.0	1.29 ± 0.10	8
75B23	B10	6.9	8.50 ± 0.21	8
27K02	S2348	9.6	11.57 ± 0.30	8
50G10	R2174	13.5	14.31 ± 0.29	8
E30A10	G1125	15.4	20.71 ± 0.44	8
	pRCS2	Centromere	23.01 ± 0.38	9
53D03	C489	15.9	24.18 ± 0.34	8
32P12	C148	17.5	28.59 ± 0.62	10
50N08	S1786	24.3	39.39 ± 0.54	10
61K21	S10886	31.7	43.31 ± 0.42	8
05G15	R2604	41.0	52.66 ± 0.33	8
93B11	S11014	47.1	58.17 ± 0.43	8
41P03	R1877	52.8	61.90 ± 0.40	8
E18B10	C488	58.4	70.43 ± 0.29	8
E15I11	C16	71.2	73.11 ± 0.67	8
E14I11	C797	72.6	75.34 ± 0.29	8
03O19	C239	82.9	82.75 ± 0.04	8
56G17	C405	83.7	83.70 ± 0.00	8

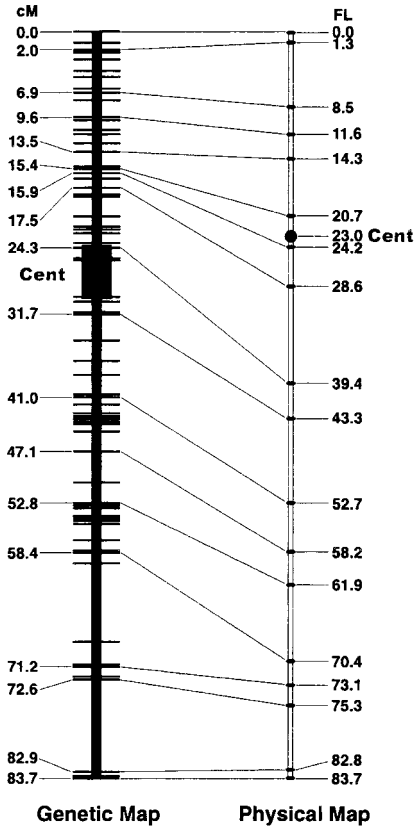

FIGURE 4.—Comparison of the genetic linkage map and pachytene FISH-based physical map of rice chromosome 10. The linkage map was drawn according to HARUSHIMA *et al.* (1998). The position of each BAC on the pachytene chromosome is calculated as the fractional length (FL; see MATERIALS AND METHODS).

KÜNZEL *et al.* 2000). In addition, most markers are tightly clustered in small-sized physical segments (GILL *et al.* 1996; FARIS *et al.* 2000; KÜNZEL *et al.* 2000). Significant suppression of genetic recombination in the pericentromeric regions was also reported in tomato (TANKSLEY *et al.* 1992). SHERMAN and STACK (1995) demonstrated that recombination nodules are rarely observed in the proximal half of all the 12 individual synaptonemal complexes in tomato.

A YAC-based contig map and complete sequencing of chromosome 4 of *A. thaliana* allowed a thorough comparison of genetic and physical distances (SCHMIDT *et al.* 1995; MAYER *et al.* 1999). The ratio of physical to genetic distance between markers varies substantially along the length of the chromosome, with the average value being 185 kb/cM. Relative hot spots (30–50 kb/cM) and cold spots (>550 kb/cM) were distributed throughout the chromosome (SCHMIDT *et al.* 1995). Recombination suppression in the pericentromeric region was not as evident as that reported in tomato, wheat, and barley chromosomes.

Variation of the ratio of physical to genetic distance between markers has also been reported in rice. Genetic mapping of the ends of large-size YAC clones suggests that the physical distance corresponding to 1 cM varies from 120 to 1000 kb, depending on the chromosomal region (UMEHARA *et al.* 1994). A high recombination rate (<50 kb/cM) was observed at the distal ends of chromosomes 9 and 11 using pulsed-field gel electrophoresis (WU and TANKSLEY 1993). It was suggested that the inhibition of meiotic recombination is limited to the narrow regions around the rice centromeres because the values of physical length per centimorgan are

not significantly higher than average in several regions within 5 cM from the centromere (Harushima *et al.* 1998). In this study, we demonstrate that significant recombination suppression (7-fold reduction) in the centromere is restricted within a relatively small region, ~4% of the physical size of chromosome 10. A 3.5-fold recombination reduction is located within the centromeric region (from 13.5 to 17.5 cM) that corresponds to 17% of the physical size of chromosome 10. Thus, the degree of recombination suppression in the centromeric region of rice chromosome 10 is similar to that of chromosome 4 of *A. thaliana*, but significantly different from those reported in wheat and barley.

The region between markers C488 (58.4 cM) and C16 (71.2 cM) shows a higher rate of recombination than the rest of the regions of chromosome 10 (Figure 4). Besides this region and the centromeric region, significant variation in the ratio of physical to genetic distance between markers is not observed in rice chromosome 10. Hot and/or cold spots restricted within much smaller regions may exist but were not discovered in the present study because the current physical map consists of only a limited number of RFLP marker-anchored BAC clones. In general, the relationship between genetic and physical distance along rice chromosome 10 resembles that of chromosome 4 of *A. thaliana* and differs from those reported in several plant species with large genomes, including tomato, wheat, and barley. One notable result in this study is that the short arm of chromosome 10 shows a relatively lower recombination frequency (0.74 cM/FL) than the long arm (1.14 cM/FL). This is correlated with the fact that the short arm is more heterochromatic than the long arm on the basis of the chromosomal staining patterns using acetocarmine (Cheng and Gu 1994).

Utility of FISH-based physical mapping in rice genome sequencing: Rice is the first grass species to be completely sequenced (Sasaki and Burr 2000). The rice genome is only three times larger than that of *A. thaliana* (140 Mb). However, the rice genome contains a higher proportion of heterochromatic chromosomal regions than *A. thaliana* on the basis of the DAPI staining of pachytene chromosomes (Fransz *et al.* 1998; Cheng *et al.* 2001). Thus, rice genome sequencing is potentially much more challenging than sequencing of the *A. thaliana* genome. The genetic linkage map of rice reported by Harushima *et al.* (1998) contains more markers per centimorgan than any other plant genetic map. However, it still contains 39 gaps that span >5 cM, including two such gaps in the distal regions on the long arm of chromosome 10 (Figure 4). Our results indicate that at least some of these gaps, such as the one spanning 72.6–82.9 cM on chromosome 10 (Figure 4), are not associated with recombination hot spots and represent a large chromosomal segment. These gaps may be problematic to close because of the scarcity of DNA markers in these regions.

FISH-based physical mapping will be a valuable complement to the ongoing rice genome sequencing project. First, the chromosomal locations of uncertain BAC clones or contigs can be unambiguously assigned to specific chromosomes by FISH analysis on somatic metaphase chromosomes with the aid of chromosome-specific FISH markers (Cheng *et al.* 2001). Second, pachytene FISH can be used to determine if the linkage gaps represent recombination hot spots or large chromosomal segments. Such information will be valuable for designing corresponding strategies to eventually close gaps. Third, pachytene FISH and fiber-FISH can be used to estimate the physical distance between the telomere and BAC clones located at distal regions. The tandem repeat Os48 is located at nine different distal ends of eight rice chromosomes (Cheng *et al.* 2001) and can be used for fiber-FISH analysis as demonstrated in the present study. If a distal BAC is <30–50 kb away from the telomere, the telomeric $(TTTAGGG)_n$ sequence could also be used as a terminal DNA reference marker for fiber-FISH analysis. Fourth, fiber-FISH analysis can be used to estimate contig and/or sequencing gaps using clones that flank the gaps (Jackson *et al.* 1998, 2000). This approach has been used to gauge the physical sizes of the majority of the sequencing gaps in human chromosomes 21 and 22 (Dunham *et al.* 1999; Hattori *et al.* 2000). Fiber-FISH analysis of individual BAC molecules (Jackson *et al.* 1999) can be used to position specific subclones within a BAC insert and to assist in assembly of highly repetitive sequences.

This research was partially supported by a grant from the Consortium for Plant Biotechnology Research, Inc., Novartis Seeds, Inc., and Dow AgroSciences.

LITERATURE CITED

Arumuganathan, K., and E. D. Earle, 1991 Nuclear DNA content of some important plant species. Plant Mol. Biol. Rep. **9:** 208–218.

Causse, M. A., T. M. Fulton, Y. G. Cho, S. N. Ahn, J. Chunwongse *et al.*, 1994 Saturated molecular map of the rice genome based on an interspecific backcross population. Genetics **138:** 1251–1274.

Chen, C. C., C. M. Chen, F. C. Hsu, C. J. Wang, J. T. Yang *et al.*, 2000 The pachytene chromosomes of maize as revealed by fluorescence *in situ* hybridization with repetitive DNA sequences. Theor. Appl. Genet. **101:** 30–36.

Cheng, Z. K., and M. H. Gu, 1994 Karyotype analysis for pachytene chromosome of *indica*, *japonica* rice and their hybrid. Chinese J. Genet. **21:** 182–187.

Cheng, Z. K., H. X. Yu, C. J. Yan, L. H. Zhu and M. H. Gu, 1997 Cytological identification of an isotetrasomic in rice and its application to centromere mapping. Cell Res. **7:** 31–38.

Cheng, Z. K., R. M. Stupar, M. H. Gu and J. Jiang, 2001 A tandemly repeated DNA sequence is associated with both knob-like heterochromatin and a highly decondensed structure in the meiotic pachytene chromosomes of rice. Chromosoma (in press).

De Jong, J. H., P. Fransz and P. Zabel, 1999 High resolution FISH in plants—techniques and applications. Trends Plant Sci. **4:** 258–263.

Delaney, D., S. Nasuda, T. R. Endo, B. S. Gill and S. H. Hulbert, 1995a Cytogenetically based physical maps of the group-2 chromosomes of wheat. Theor. Appl. Genet. **91:** 568–573.

Delaney, D., S. Nasuda, T. R. Endo, B. S. Gill and S. H. Hulbert,

1995b Cytogenetically based physical maps of the group-3 chromosomes of wheat. Theor. Appl. Genet. **91:** 780–782.

Dong, F., J. T. Miller, S. A. Jackson, G. L. Wang, P. C. Ronald *et al.*, 1998 Rice (*Oryza sativa*) centromeric regions consist of complex DNA. Proc. Natl. Acad. Sci. USA **95:** 8135–8140.

Dong, F., J. Song, S. K. Naess, J. P. Helgeson, C. Gebhardt *et al.*, 2000 Development and applications of a set of chromosome-specific cytogenetic DNA markers in potato. Theor. Appl. Genet. **101:** 1001–1007.

Dunham, I., N. Shimizu, B. A. Roe, S. Chisose, I. Dunham *et al.*, 1999 The DNA sequence of human chromosome 22. Nature **402:** 489–495.

Faris, J. D., K. M. Haen and B. S. Gill, 2000 Saturation mapping of a gene-rich recombination hot spot region in wheat. Genetics **154:** 823–835.

Fransz, P. F., C. Alonso-Blanco, T. B. Liharska, A. J. M. Peeters, P. Zabel *et al.*, 1996 High-resolution physical mapping in *Arabidopsis thaliana* and tomato by fluorescence *in situ* hybridization to extended DNA fibers. Plant J. **9:** 421–430.

Fransz, P. F., S. Armstrong, C. Alonso-Blanco, T. C. Fischer, R. A. Torres-Ruiz *et al.*, 1998 Cytogenetics for the model system *Arabidopsis thaliana*. Plant J. **13:** 867–876.

Fransz, P. F., S. Armstrong, J. H. de Jong, L. D. Parnell, G. van Drunen *et al.*, 2000 Integrated cytogenetic map of chromosome arm 4S of *A. thaliana*: structural organization of heterochromatic knob and centromere region. Cell **100:** 367–376.

Fukui, K., N. Ohmido and G. S. Khush, 1994 Variability in rDNA loci in the genus *Oryza* detected through fluorescence *in situ* hybridization. Theor. Appl. Genet. **87:** 893–899.

Gerlach, W. L., and J. R. Bedbrook, 1979 Cloning and characterization of ribosomal RNA genes from wheat and barley. Nucleic Acids Res. **7:** 1869–1885.

Gill, K. S., B. S. Gill and T. R. Endo, 1993 A chromosome region-specific mapping strategy reveals gene-rich telomeric ends in wheat. Chromosoma **102:** 374–381.

Gill, K. S., B. S. Gill, T. R. Endo and T. Taylor, 1996 Identification and high-density mapping of gene-rich regions in chromosome group 1 of wheat. Genetics **144:** 1883–1891.

Goff, S. A., 1999 Rice as a model for cereal genomes. Curr. Opin. Plant Biol. **2:** 86–89.

Harushima, Y., M. Yano, A. Shomura, M. Sato, T. Shimano *et al.*, 1998 A high-density rice genetic linkage map with 2275 markers using a single F_2 population. Genetics **148:** 479–494.

Hattori, M., A. Fujiyama, T. D. Taylor, H. Watanabe, T. Yada *et al.*, 2000 The DNA sequence of human chromosome 21. Nature **405:** 311–319.

Jackson, S. A., M. L. Wang, H. M. Goodman and J. Jiang, 1998 Application of Fiber-FISH in genome analysis of *Arabidopsis thaliana*. Genome **41:** 566–572.

Jackson, S. A., F. Dong and J. Jiang, 1999 Digital mapping of bacterial artificial chromosomes by fluorescence *in situ* hybridization. Plant J. **17:** 581–587.

Jackson, S. A., Z. K. Cheng, M. L. Wang, H. M. Goodman and J. Jiang, 2000 Comparative fluorescence in situ hybridization mapping of a 431-kb *Arabidopsis thaliana* bacterial artificial chromosome contig reveals the role of chromosomal duplications in the expansion of the *Brassica rapa* genome. Genetics **156:** 833–838.

Jiang, J., and B. S. Gill, 1994 Non-isotopic *in situ* hybridization and plant genome mapping: the first ten years. Genome **37:** 717–725.

Jiang, J., B. S. Gill, G. L. Wang, P. C. Ronald and D. C. Ward, 1995 Metaphase and interphase fluorescence in situ hybridization mapping of the rice genome with bacterial artificial chromosomes. Proc. Natl. Acad. Sci. USA **92:** 4487–4491.

Jiang, J., S. H. Hulbert, B. S. Gill and D. C. Ward, 1996 Interphase fluorescence *in situ* hybridization mapping: a physical mapping strategy for plant species with large complex genomes. Mol. Gen. Genet. **252:** 497–502.

Künzel, G., L. Korzun and A. Meister, 2000 Cytologically integrated physical restriction fragment length polymorphism maps for the barley genome based on translocation breakpoints. Genetics **154:** 397–412.

Kurata, N., Y. Umehara, H. Tanoue and T. Sasaki, 1997 Physical mapping of the rice genome with YAC clones. Plant Mol. Biol. **35:** 101–113.

Mao, L., T. C. Wood, Y. Yu, M. A. Budiman, J. Tomkins *et al.*, 2000 Rice transposable elements: a survey of 73,000 sequence-tagged-connectors. Genome Res. **10:** 982–990.

Mayer, K., C. Schüller, R. Wambutt, G. Murphy, G. Volckaert *et al.*, 1999 Sequence and analysis of chromosome 4 of the plant Arabidopsis thaliana. Nature **402:** 769–777.

Mozo, T., K. Dewar, P. Dunn, J. R. Ecker, S. Fischer *et al.*, 1999 A complete BAC based physical map of the *Arabidopsis thaliana* genome. Nat. Genet. **22:** 271–275.

Peterson, D. G., N. L. V. Lapitan and S. M. Stack, 1999 Localization of single- and low-copy sequences on tomato synaptonemal complex spreads using fluorescence *in situ* hybridization (FISH). Genetics **152:** 427–439.

Richards, E. J., and F. M. Ausubel, 1988 Isolation of a higher eukaryotic telomere from *Arabidopsis thaliana*. Cell **53:** 127–136.

Riera-Lizarazu, O., M. I. Vales, E. V. Ananiev, H. W. Rines and R. L. Phillips, 2000 Production and characterization of maize chromosome 9 radiation hybrids derived from an oat-maize addition line. Genetics **156:** 327–339.

Sasaki, T., and B. Burr, 2000 International Rice Genome Sequencing Project: the effort to completely sequence the rice genome. Curr. Opin. Plant Biol. **3:** 138–141.

Schmidt, R., J. West, K. Love, Z. Lenehan, C. Lister *et al.*, 1995 Physical map and organization of *Arabidopsis thaliana* chromosome 4. Science **270:** 480–483.

Sherman, J. D., and S. M. Stack, 1995 Two-dimensional spreads of synaptonemal complexes from solanaceous plants. VI. High-resolution recombination nodule map for tomato (*Lycopersicon esculentum*). Genetics **141:** 683–708.

Singh, K., T. Ishii, A. Parco, N. Huang, D. S. Brar *et al.*, 1996 Centromere mapping and orientation of the molecular linkage map of rice (*Oryza sativa* L.). Proc. Natl. Acad. Sci. USA **93:** 6163–6168.

Song, J., F. Dong and J. Jiang, 2000 Construction of a bacterial artificial chromosome (BAC) library for potato molecular cytogenetics research. Genome **43:** 199–204.

Tanksley, S. D., M. W. Ganal, J. P. Prince, M. C. de Vicente, M. W. Bonierbale *et al.*, 1992 High density molecular linkage maps of the tomato and potato genomes. Genetics **132:** 1141–1160.

Umehara, Y., A. Inagaki, H. Tanoue, Y. Yasukochi, Y. Nagamura *et al.*, 1994 Construction and characterization of a rice YAC library for physical mapping. Mol. Breed. **1:** 78–89.

Wang, S., J. Wang, J. Jiang and Q. Zhang, 2000 Mapping of centromeric regions on the molecular linkage map of rice (*Oryza sativa* L.) using centromere-associated sequences. Mol. Gen. Genet. **263:** 165–172.

Weber, D., and T. Helentjaris, 1989 Mapping RFLP loci in maize using B-A translocations. Genetics **121:** 583–590.

Werner, J. E., T. R. Endo and B. S. Gill, 1992 Toward a cytogenetically based physical map of the wheat genome. Proc. Natl. Acad. Sci. USA **89:** 11307–11311.

Wu, K.-S., and T. D. Tanksley, 1993 Genetic and physical mapping of telomeres and macrosatellites of rice. Plant Mol. Biol. **22:** 861–872.

Wu, T., and R. Wu, 1987 A new rice repetitive DNA sequence shows homology to both 5s RNA and tRNA. Nucleic Acids Res. **15:** 5913–5923.

Wu, T., Y. Wang and R. Wu, 1994 Transcribed repetitive DNA sequences in telomeric regions of rice (*Oryza sativa*). Plant Mol. Biol. **26:** 363–375.

Xu, J., and E. D. Earle, 1996 High resolution physical mapping of 45S (5.8S, 18S and 25S) rDNA gene loci in the tomato genome using a combination of karyotyping and FISH of pachytene chromosomes. Chromosoma **104:** 545–550.

Zhong, X. B., J. Bodeau, P. F. Fransz, V. M. Williamson, A. van Kammen *et al.*, 1999 FISH to meiotic pachytene chromosomes of tomato locates the root knot nematode resistance gene *Mi-1* and the acid phosphatase gene *Aps-1* near the junction of euchromatin and pericentromeric heterochromatin of chromosome arms 6S and 6L, respectively. Theor. Appl. Genet. **98:** 365–370.

Zwick, M. S., M. N. Islam-Faridi, D. G. Czechin, Jr., R. A. Wing, G. E. Hart *et al.*, 1998 Physical mapping of the liguleless linkage group in Sorghum bicolor using rice selected RFLP Sorghum BACs. Genetics **148:** 1983–1992.

Communicating editor: B. S. Gill