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ORIGINAL PAPER

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An integrated map of *Oryza sativa* L. chromosome 5

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Abstract The developments of molecular marker-based genetic linkage maps are now routine. Physical maps based on contigs of large insert genomic clones have been established in several plant species. However, integration of genetic, physical, and cytological maps is still a challenge for most plant species. Here we present an integrated map of rice (*Oryza sativa* L.) chromosome 5, developed by fluorescence in situ hybridization mapping of 18 bacterial artificial chromosome (BAC) clones or PI-derived artificial chromosome (PAC) clones on meiotic pachytene chromosomes. Each BAC/PAC clone was anchored by a restriction fragment length polymorphism marker mapped to the rice genetic linkage map. This molecular cytogenetic map shows the genetic recombination and sequence information of a physical map, correlated to the cytological features of rice chromosome 5. Detailed comparisons of the distances between markers on genetic, cytological, and physical maps, revealed the distributions of recombination events and molecular organization of the chromosomal features of rice chromosome 5 at the pachytene stage. Discordance of distances between the markers was found among the different maps. Our results revealed that neither the recombination events nor the degree of chromatin condensation were evenly distributed along the entire length of chromosome 5. Detailed comparisons of the correlative positions of markers on the genetic, cytological, and physical maps of rice chromosome 5 provide insight into the molecular architecture of rice chromosome 5, in relation to its cytological features and recombination events on the genetic map. The prospective applications of such an integrated cytogenetic map are discussed.

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Introduction

The structure and function of the rice genome has been extensively investigated for several decades since Kuwada (1910) determined the rice chromosome number $2n = 24$, and Nagao and Takahashi (1963) reported the first genetic linkage map of rice. Primary trisomics (Iwata and Omura 1976; Khush et al. 1984) and reciprocal translocation lines (Nishimura 1961; Chung and Wu 1994) have been established and extensively used for the determination of the corresponding relationships of gene-chromosome-linkage group in rice genome. A compiled rice genetic linkage map was accomplished by Kinoshita (1990). It accumulated knowledge on genetic analyses of about 300 rice genes of morphological traits and isozyme markers in a period of 50 years (1940–1990) reported in 224 papers (Khush 1990). Abundant genetic knowledge and intensive plant breeding have promoted rice as a model for cereal genomics (for review, see Havukkala 1996; Goff 1999). The restriction fragment length polymorphism (RFLP)-based genetic maps of rice are among the most saturated maps in plant species (Causse et al. 1994; Harushima et al. 1998). A physical map with high coverage bacterial artificial chromosome (BAC)/PI-derived artificial chromosome (PAC) contigs, spanning whole rice genome is available (Chen et al. 2002). Most of these BACs/PACs were anchored with RFLP markers on a genetic map by DNA gel blot hybridization in silico comparison (Chen et al. 2002).

Drafts of the rice genome sequence are publicly available (Sasaki and Burr 2000; Goff et al. 2002; Yu et al. 2002). In addition, phase 3 level finished sequence of chromosomes 1, 3, 4, 5, 10, 11, and 12 have already been reported by scientists from International Rice Genome Sequencing Project (IRGSP) consortium (Feng et al. 2002; Sasaki et al. 2002; Rice Chromosome 10 Sequencing Consortium 2003; Rice Chromosomes 11 and 12 Sequencing Consortia 2005; Yu et al. 2005; Buell et al. 2005; Cheng et al. 2005). The finished-quality sequence, which covers 95% of the 389 Mb genome, has been presented (International Rice Genome Sequencing Project 2005).

Despite the extensive research of the rice genome in the last decade, our knowledge of the chromosomal organization at the molecular level has lagged behind, when compared to the rapid acquisition of genomic DNA sequence information (Lam et al. 2004). Thus, it is important to create a cytogenetic map showing the relationship between recombination frequency (genetic map) and cytological features of chromosomal organization (cytological map) at the DNA level (physical map). The establishment of corresponding relationship between cytological map and genetic map requires a saturated genetic map, a well-developed system for cytological analysis, different types of cytogenetic stocks, such as trisomics, translocation lines, and deletion stocks (Harper and Cande 2000). Now it is possible to integrate cytogenetic maps with physical maps, which were generated based on extended DNA sequence database, by fluorescent in situ hybridization (FISH) method. Some examples have been reported in plant species, such as rice chromosome 10 (Cheng et al. 2001b) and *Sorghum bicolor*, L. chromosome 1 (Islam-Faridi et al. 2002).

Many efforts had been attempted to improve techniques of rice chromosome preparation (Shastry et al. 1960; Wu 1967; Kurata and Omure 1978; Chung and Wu 1987; Fukui and Iijima 1991). Furthermore, a set of 24 chromosomal arm-specific BAC clones has been evaluated to facilitate rice chromosome identification (Cheng et al. 2001a). A fine physical map of chromosome 5 of *Oryza sativa* ssp. *japonica* var. Nipponbare, with BAC/PAC clones spanning 30.02 Mb, has been constructed by Academia Sinica Plant Genome Center (Cheng et al. 2005). These resources provide an excellent foundation to develop a fully integrated map of rice chromosome 5, which would address the physical position of the rice genes, and the precise relationship between the cytological features of a chromosome and its genetic map. To achieve this goal, 18 BACs/PACs anchored with RFLP markers on the genetic map were FISH-mapped to meiotic pachytene chromosome spreads to assign their cytological locations on rice chromosome 5. We established an integrated molecular cytogenetic map of rice chromosome 5, which reveals the cytological positions of RFLP marker-anchored BAC/PAC clones. Detailed comparisons of the distances between the markers on the genetic, cytological, and physical maps of rice chromosome 5 provide insight into the molecular architecture of rice chromosome 5, in relation to its cytological features and recombination events on the genetic map.

Materials and methods

Materials

Oryza sativa ssp. *Japonica* rice variety Nipponbare was used for BAC/PAC–FISH mapping on rice chromosomes. All BAC/PAC clones used in this study were identified by screening Nipponbare BAC libraries or PAC libraries (http://www.genome.clemson.edu/orders/product.html) with RFLP markers previously mapped to the rice chromosome 5 genetic map (Harushima et al. 1998; Oryzabase: http://www.shigen.lab.nig.ac.jp) and were used to construct the pseudomolecule (physical map) of rice chromosome 5 (Cheng et al. 2005). Eighteen clones that were evenly distributed along the entire length of the physical map of rice chromosome 5 were selected and used as FISH probes (Table 1). The nomenclature of clones followed the descriptions of Clemson University Genomic Institute (CUGI, http://www.genome.clemson.edu). Plasmid pRCS2 (Dong et al. 1998), containing rice centromere-specific CentO repeats, was used to identify the centromere position on the rice chromosomes.

Fluorescence in situ hybridization

Young panicles of rice were fixed with 95% ethanol and glacial acetic acid (3:1) overnight, then stored at −20°C. Fixed materials can be stored for several months; however, cytoplasm of microsporocytes may become dense during storage. Microsporocytes at the pachytene stage were squashed in 45% acetic acid then stored at −70°C until required. In our experience, chromosome preparations remain qualified for FISH experiments after 1-year storage at −70°C. FISH was performed as described by Jiang et al. (1995) and Cheng et al. (2001b) with few modifications. The chromosome preparations were denatured in 70% formamide/2× SSC at 80°C for 90 s, immersed into ice-cold 75% ethanol immediately, dehydrated in a ethanol series, and air-dried before being incubated with FISH probes cocktail. To permit the simultaneous identification of numerous sites of clones on a single chromosome, pachytene chromosome preparations were hybridized to a two-hapten FISH probe cocktail with probes derived from multiple BACs/PACs and pRCS2. Each slide was applied with 20 µl hybridization mixture containing 50% deionized formamide, 2× SSC, 10% dextran sulfate, 50–100 ng labeled probes for each target and 20 µg salmon sperm sheared DNA as suppressive blocking DNA. Unlabeled C_0t-1 DNA was not included in the hybridization mixture in this study. Hybridization was performed overnight in a moist chamber at 37°C. Probes were labeled with either

Table 1 Genetic, cytological, and physical locations of RFLP markers and their corresponding BAC clones

Clone	Marker	cM[a]	CF[b]	PF[c]	Accession
P0036D10	–	0–3	0.00	0.37	AC073405
P0033D06	R3166	19	6.86 ± 2.01	7.25	AC079357
OJ1725E07	E749S	36.4	26.58 ± 2.80	21.10	AC093920
b1007D10	S10613S	38.3	27.14 ± 1.32	21.85	AC148611
b0012L23	E11511S	47.2	29.52 ± 1.06	28.93	AC137001
P0499F10	R3238	49.4	31.38 ± 2.63	31.23	AC132487
a0042F15	E3093S	51.3	31.38 ± 2.63	31.74	AC145396
a0018H09	–	54.3–54.6	37.37 ± 1.24	45.44	AC137610
P0697B04	S20487S	54.6[d]	42.17 ± 0.68	50.49	AC137984
b1023E01	–	54.6–55.4	45.58 ± 0.72	55.62	AC145476
a0077J17	C60228S	60.7	52.66 ± 1.32	67.32	AC136221
a0009L15	E2801S	64.1	56.39 ± 3.57	69.56	AC136999
b0006J12	C2269S	75	69.91 ± 0.65	80.01	AC120991
OJ1058F05	R594	87.4	83.44 ± 1.27	87.04	AC105318
b0042J17	C11282S	87.4	83.94 ± 1.71	87.34	AC105318
OJ1123F01	L176	103.9	95.26 ± 0.70	101.94	AC113334
OJ1345B12	R3085	110.7	109.83 ± 1.15	110.63	AC104278
a0035I01	–	122.3	122.30	121.84 122.30	AC137000

[a]CentiMorgans (cM) are based on the percentage of recombination between markers on RFLP map of rice chromosome 5 (Harushima et al. 1998). The total length of each map was divided into 122.3 fractions
[b]Cytological fraction (CF) position of BACs/PACs are calculated according to probes derived FISH signals on pachytene chromosomes
[c]Physical fraction (PF) position of BACs/PACs are calculated according to their position on pseudomolecule of rice chromosome 5 (ASPGC)
[d]The centromere position at genetic map of rice chromosome 5

biotin-16-dUTP or digoxigenin-11-dUTP by nick translation following the manufacturer's protocol (Roche Diagnostics GmbH, Penzberg Germany). Hybridization sites were immunologically detected by using either fluorescein isothiocyanate (FITC)-conjugated avidin (Vector Laboratories, CA, USA) for biotin-labeled probes or a rhodamine-conjugated anti-digoxigenin antibody for Dig-labeled probes (Roche Diagnostics GmbH), respectively. Chromosomes were counterstained with 4′, 6-diamidino-2-phenylindole (DAPI) in an antifade solution (Vector Laboratories). Chromosome preparations were examined and photographed with an epifluorescence microscope (Axioplan, Carl Zeiss AG, Jena, Germany) and a CoolSanp-*fx* charged-coupled device camera (Photometrics, Tucson, USA). Images caught by the camera were pseudocolored and combined using Image-Pro Plus software (V5.0.2.9, Media Cybenetics Inc., USA). Final image adjustments were done with Adobe Photoshop 6.0 software (Adobe Systems Incorporated, USA).

Measurement and calculation of fractional position on the map

Chromosomes bearing clear FISH signals were photographed and analyzed. The total length of chromosomes and the distance from the FISH signals to the distal end of the short arm of the chromosome were digitally measured in arbitrary units, using Image-Pro Plus software (V5.0.2.9, Media Cybenetics Inc.). A minimum of five pachytene chromosome images with good quality was analyzed.

The total centiMorgans (cM) value of the RFLP linkage map of rice chromosome 5 has been estimated as 122.3 cM (Harushima et al. 1998). For convenient comparison, the distance between the clones on different maps and the position of each clone was calculated as a fraction of the total length of the map. The total length of each map was divided into 122.3 fractions. The cytological fractional (CF) position of a BAC/PAC on a pachytene chromosome (cytological map) was calculated as $(L_X/L_T)\times 122.3$, where L_X was the length (in micrometer, μm) of the segment between the FISH hybridization site and the distal end of the short arm of the chromosome, and L_T was the total length of the chromosome (in μm). The physical fractional (PF) position of a BAC/PAC on a pseudomolecule of rice chromosome 5 (physical map) was calculated as $(M_X/M_T)\times 122.3$; M_X was the physical size (in megabase, Mb) of the fragment between the position of the BAC clone and the north end of the chromosome 5 pseudomolecule; M_T was the physical size of the entire length of rice chromosome 5 in megabase pairs, 30.02 Mb. The fractional positions of BACs/PACs on the individual maps are summarized in Table 1.

Results

Features of rice chromosome 5

The chromosomal features of rice chromosome 5 at the pachytene stage observed in this study are similar to previous descriptions revealed by acetocarmine staining (Chung and Wu 1987) and DAPI staining (Cheng et al.

2001a). Chromosome 5 can be identified, based on relative length, centromere position, and DAPI-stained patterns from pachytene complements. Chromosome 5, like most of the other rice chromosomes, has notable pericentromeric heterochromatin. The average length was estimated as 46.49 ± 7.25 μm based on 30 images. In general, the heterochromatin was stained brightly with DAPI. The proximal region on the short arm of chromosome 5 represents the most heterochromatic region on rice chromosome 5, while its long arm is comparatively euchromatic (Fig. 1). The knob-like heterochromatin, a brightly DAPI stained structure, was observed at the distal end of the long arm of chromosome 5 of Nipponbare (Fig. 1) Each image, shown in Figs. 1, 2, 3, and 4, was the result of one FISH experiment, with a mixture of probes prepared from multiple BAC/PACs, labeled with digoxigenin-11-dUTP and detected by rhodamine-conjugated anti-digoxigenin antibody (red), and from plasmid pRCS2, labeled with biotin-16-dUTP and detected by fluorescein isothiocyanate (FITC)-conjugated avidin (green).

The FISH signals, derived from the clones P0036D10 (0–3 cM) and a0035I01 (122.3 cM), were observed at the distal ends of both arms of pachytene chromosome 5 (Fig. 2a, b). These results showed that the current genetic linkage map well span the entire chromosome 5. We also found that the FISH signal derived from P0036D10 (0–3 cM) was detected only at the distal end of the short arm of chromosome 5 (Fig. 2c), while FISH signals derived from a0035I01 (122.3 cM) were found at the distal ends of several chromosomes, although the signals at the end of long arm of chromosome 5 were generally stronger than others. This suggests that both clone a0035I01 and the telomeric region at the long arm of rice chromosome 5 contained abundant repetitive sequences, which is parallel to the brightly DAPI-stained and knob-like heterochromatin structures observed at the distal ends of the long arm of chromosome 5 (Fig. 1).

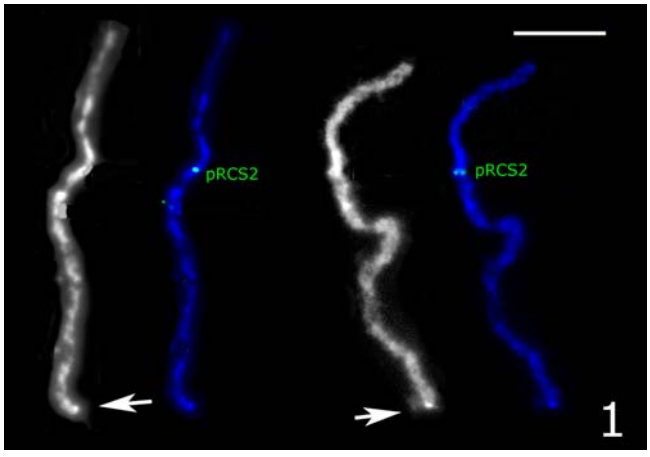

Fig. 1 The DAPI images show the rice pachytene chromosome 5. DAPI-stained pachytene chromosomes 5 (*right*) were converted to black-and-white images (*left*) to enhance visualization of the degrees of chromatin condensation of the same chromosomes. Centromeres are indicated by centromeric DNA probe pRCS2 (*green*) and the heterochromatic knob-like telomere, by *arrows* at the end of the long arm. Bar = 10 μm

P0697B04, anchored by marker S20487S at 54.6 cM (Oryzabase, http://www.shigen.lab.nig.ac.jp/rice/oryzabase), where it had been assigned as the genetic centromere position of rice chromosome 5 (Nonomura and Kurata 2001), mainly contained rice centromere-specific repeats, CentO (Cheng et al. 2005). The FISH signals derived from both pRCS2 and P0697B04 could be referred to as the precise position of the centromere on rice chromosome 5. Using the FISH signal from pRCS2 as an anchor point, the centromere was mapped at 42.17 CF on the cytological map, and the arm ratio (long arm/short arm) of rice chromosome 5 was calculated as (122.3–42.17)/42.17 = 1.90; while the position of P0697B04 on the physical map was estimated as 50.49 PF (Table 1). The average physical distance between recombination events on the short arm was calculated as 50.49 PF/54.6 cM = 0.93 PF/cM and was (122.3–50.49) PF/(122.3–54.6) cM = 1.06 PF/cM on the long arm of rice chromosome 5 (Tables 1, 2). These results indicated that the distribution of recombination on the two arms was not significantly different.

All used BACs/PACs yielded distinct FISH signals on chromosome 5 (Figs. 2, 3). The FISH results (Figs. 2, 3) allowed us to establish an integrated cytogenetic map showing the genetic map and physical map information on rice pachytene chromosome 5 (Table 1). Eight clones on the northern side of P0697B04 (54.6 cM) on the physical map were FISH-mapped on the short arm of chromosome 5, while the others were mapped on its long arm (Table 1). Based on the data listed in Table 1, the average physical distances between recombination events (PF/cM) and the average packing ratio (PF/CF) of chromatin, within defined intervals, were calculated and summarized in Table 2.

Cross hybridization of BAC/PACs to more than one site

The two clones, a0018H09 and b1023E01, flanking the centromere and delineating the genetic centromere region on rice chromosome 5, were mapped on more than one mitotic metaphase chromosome, in addition to chromosome 5, and showed intense FISH signals at the pericentromeric region of these chromosomes (Fig. 4). These results suggested that these two clones contained blocks of sequences homologous to the precentromeric DNA of several chromosomes, or contained segments duplicated within rice genome.

Uneven chromatin condensation within pericentromeric region of rice chromosome 5 at the pachytene stage

The discordance of distances between the markers found among the different maps revealed that neither the

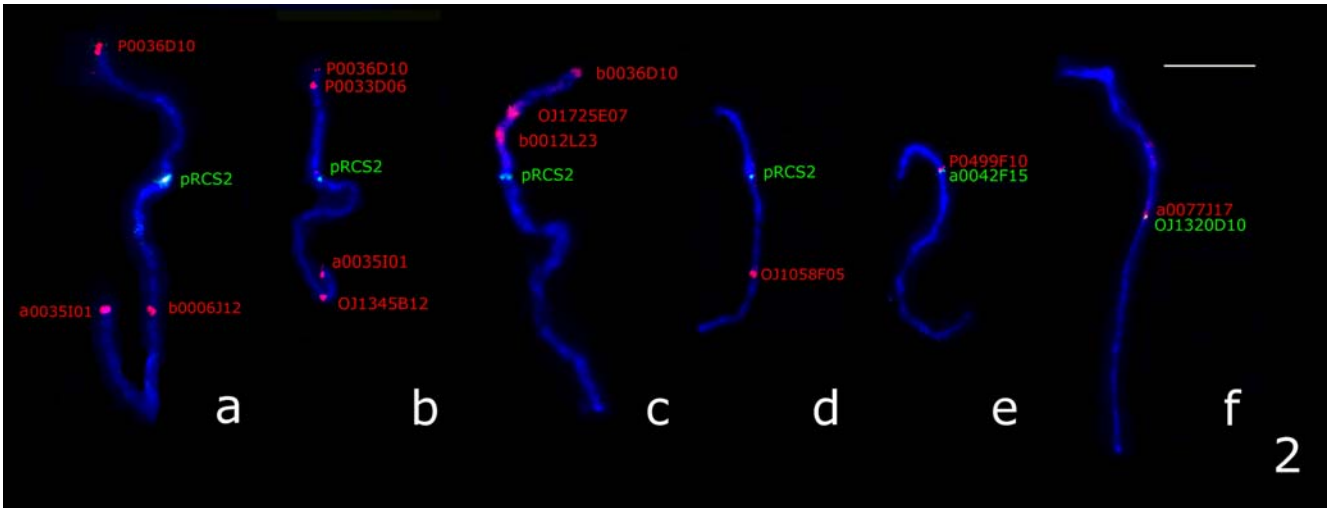

Fig. 2 Fluorescent in situ hybridization mapping of RFLP marker-anchored BACs/PACs (*red*) and centromere (*green*) on rice pachytene chromosome 5. **a** FISH mapping of three BACs/PAC clones (*red*), including two clones at the ends of both arms and one clone at the long arm, and the centromeric DNA probe pRCS2 (*green*) on the same pachytene chromosome. **b** FISH mapping of four BACs/PAC clones (*red*), including two clones at the ends of both arms and two clones near the telomeric regions of the short arm and the long arm, respectively. **c** FISH mapping of three clones at the short arm of pachytene chromosome 5. **d** FISH mapping of one BAC clone at the long arm of pachytene chromosome 5. **e**, **f** Two clone pairs, flanking physical gaps generated by genomic sequencing, were resolved using dual-colored probes. Bar = 10 μm

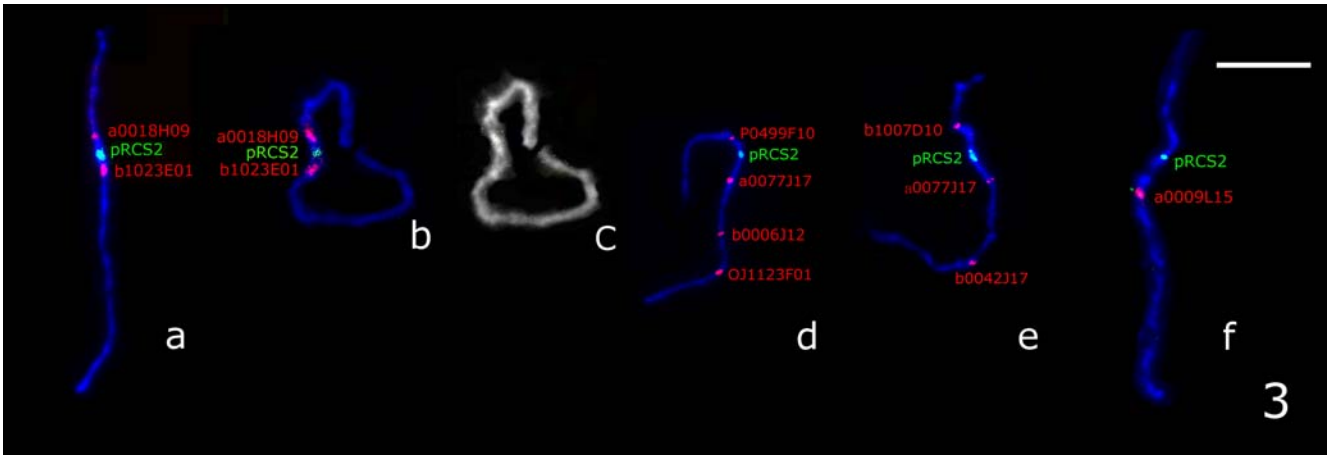

Fig. 3 Fluorescent in situ hybridization mapping of RFLP marker-anchored BACs/PACs (*red*) at the pericentromeric region on rice pachytene chromosome 5. **a**, **b** FISH mapping of two clones (*red*) flanking centromere (*green*) on rice pachytene chromosome 5. **c** DAPI-stained pachytene chromosome 5 of 2B was converted to black-and-white images to enhance visualization of the degree of chromatin condensation of the same chromosomes. **d**, **f** FISH mapping of BAC/PACs at the pericentromeric region and interstitial segments of pachytene chromosome 5. Bar = 10 μm

recombination events nor the degree of chromatin condensation were evenly distributed along the entire length of chromosome 5 (Fig. 5, Table 2). The average chromatin condensation of the short arm was calculated as 50.49 PF/42.17 CF = 1.20, according to the centromere position on the cytological map (42.17 CF) and on the physical map (50.49 PF), while it was calculated as (122.3–50.49) PF/(122.3–42.17) CF = 0.90 PF/CF for the long arm. These results suggested that the short arm chromatin may be more condensed than that of the long arm of rice chromosome 5.

The centromeric region is often the most condensed region in eukaryotic chromosomes, including rice (Cheng et al. 2001a). The chromatin within the pericentromeric region on rice chromosome 5, spanned by b1007D10 (38.3 cM, Fig. 3e) and a0077J17 (60.7 cM, Figs. 2f, 3d, e), showed various degrees of condensation (Fig. 3c, Table 2). This region could be divided into five intervals, by the cytological locations of the FISH signals derived from six probes. These intervals showed differential recombination frequency and various degrees of chromatin condensation. The PF/CF value was

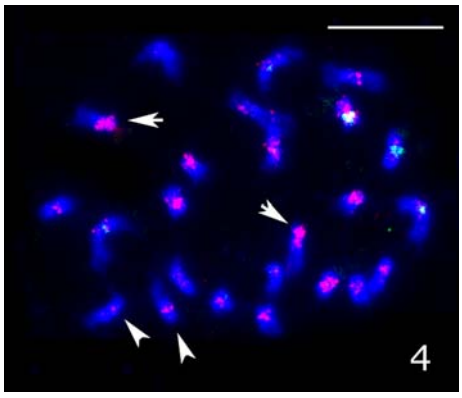

Fig. 4 Fluorescent in situ hybridization mapping of two clones, a0018H09 (*red*) and b1023E01 (*red*), flanking centromere (*green*) on rice mitotic metaphase chromosomes, showing intense FISH signals at the pericentromeric region of multiple chromosomes. *Arrows* indicate the chromosomes 4 and *arrowheads* indicate the chromosomes 5. Bar = 10 μm

calculated as 1.24 (Fig. 5, Table 2) within the genetic centromere region on the genetic map, spanned by a0018H09 (54.3–54.6 cM, Fig. 3a, b) and b1023E01 (54.6–55.4 cM, Fig. 3a, b). This interval showed the least condensation within the pericentromeric region (Fig. 3c, Table 2). The PF/CF value was calculated as 2.88 (Table 2) between a0042F15 (51.3 cM, Fig. 2e) and a0018H09 (54.3–54.6 cM, Fig. 3a, b); as 1.51 (Table 2) between clones b0012L23 (47.2 cM, Fig. 3a, b) and a0042F15 (51.3 cM, Fig. 2e); and as 2.98 (Table 2) between clones b1007D10 (38.3 cM, Fig. 3e) and b0012L23 (47.2 cM, Fig. 3a, b). This 38.3–47.2 cM interval showed the most condensation of all, on rice chromosome 5 at the pachytene stage. The PF/CF value was calculated as 1.65 (Fig. 5, Table 2) within the proximal region at the long arm between b1023E01 (54.6–55.4 cM, Fig. 3a, b) and a0077J17 (60.7 cM, Figs. 2f, 3d, e).

Recombination suppression at the pericentromeric region of rice chromosome 5

The average physical distance between the recombination events was calculated as 30.02 Mb/122.3 cM = 245 kb/cM on rice chromosome 5, and recombination frequency near the centromeric region was found to be much lower than the average value (Fig. 5, Table 2). The physical distance between a0018H09 (54.3–54.6 cM) and b1023E01 (54.6–55.4 cM), flanking the genetic centromere at 54.6 cM (Fig. 2a, b), was calculated as 10.18 PF (55.62−45.44 PF) or estimated as 2.49 Mb (245 kb×10.18 PF); approximately one recombination unit (54.3–55.4 cM) was found within this interval. The average physical size between recombination, within this region, was calculated as 9.25 PF/cM (Table 2). This suggested a dramatic repression of recombination within the genetic centromere of rice chromosome 5.

Recombination frequency was compared based on the physical distance per centiMorgan (PF/cM). The average value in the proximal region, at the short arm between a0042F15 (51.3 cM, Fig. 2e) and a0018H09 (54.3–54.6 cM, Fig. 2a, b), was calculated as 4.15 PF/cM (Tables 1, 2), which was less than half of the value

Table 2 The average physical distances between markers (PF/cM) and the average wrapped ratio (PF/CF) of chromatin on rice chromosome 5

Clone 1 (genetic position)[a]	Clone 2 (genetic position)	PF/cM[b]	PF/CF[c]
Short arm			
P0036D10 (0–3 cM)	P0697B04 (54.6 cM)	0.93	1.20
P0036D10 (0–3 cM)	P0033D06 (19 cM)	0.36	1.00
P0036D10 (0–3 cM)	OJ1725E07 (36.4 cM)	0.57	0.78
P0033D06 (19 cM)	OJ1725E07 (36.4 cM)	0.80	0.70
Pericentromeric region			
b1007D10 (38.3 cM)	b0012L23 (47.2 cM)	0.80	2.98
b0012L23 (47.2 cM)	a0042F15 (51.3 cM)	0.68	1.51
a0042F15 (51.3 cM)	a0018H09 (54.3–54.6 cM)	4.15	2.88
a0018H09 (54.3–54.6 cM)	b1023E01 (54.6–55.4 cM)	9.25	1.24
b1023E01 (54.6–55.4 cM)	a0077J17 (60.7 cM)	1.92	1.65
Long arm			
P0697B04 (54.6 cM)	a0035I01 (122.3 cM)	1.06	0.90
a0077J17 (60.7 cM)	a0035I01 (122.3 cM)	0.89	1.27
a0077J17 (60.7 cM)	a0009L15 (64.1 cM)	0.66	0.60
a0009L15 (64.1 cM)	b0006J12 (75 cM)	0.96	0.77
b0006J12 (75 cM)	OJ1058F05 (87.4 cM)	0.72	0.52
OJ1058F05 (87.4 cM)	OJ1123F01 (103.9 cM)	0.78	1.26
OJ1123F01 (103.9 cM)	OJ1345B12 (110.7 cM)	1.28	0.60
OJ1345B12 (110.7 cM)	a0035I01 (122.3 cM)	0.97	0.90

[a]Genetic position, cM: the position of markers on genetic map
[b]PF/cM: average physical size between genetic recombination within the segment separated by clone 1 and clone 2 on rice chromosome 5
[c]PF/CF: Average physical size wrapped into a fractional length of pachytene chromosome within the segment separated by clone 1 and clone 2 on rice chromosome 5

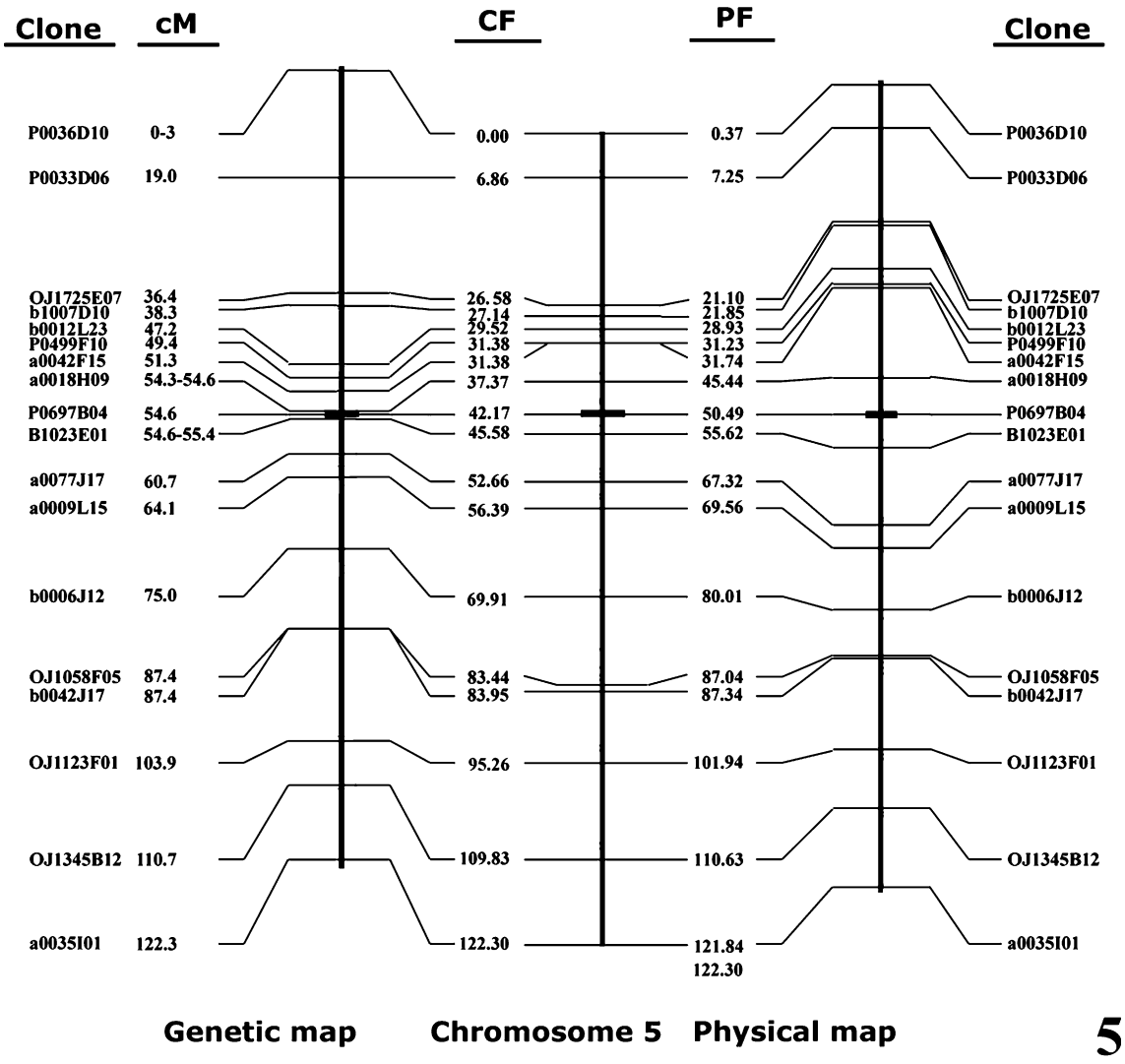

Fig. 5 The integrated cytogenetic map of rice chromosome 5, showing information from the genetic and physical maps of rice chromosome 5. CentiMorgans (*cM*) are based on the percentage of recombination between the markers on the RFLP map of rice chromosome 5 (Harushima et al. 1998). The total length of each map was divided into 122.3 fractions. The cytological fraction (*CF*) position of BACs/PACs were calculated, according to probe-derived FISH signals on the pachytene chromosomes. The physical fraction (*PF*) positions of BACs/PACs were calculated according to their position on the pseudomolecule of rice chromosome 5 (Cheng et al. 2005)

associated with the genetic centromere region. Clones a0077J17 (60.7 cM, Figs. 2f, 3d, e) and b1023E01 (54.6–55.4 cM, Fig. 3a, b) at the pericentromeric region of the long arm were genetically separated by 6.1 cM, or physically separated by 11.7 PF (Fig. 5, Table 1). The average physical distance between the above markers was 1.92 PF/cM, suggesting that recombination frequency was much greater than that in the centromere region (54.3–55.4 cM) or in the proximal region (51.3–54.6 cM) on the short arm. The interval between clones b0012L23 (47.2 cM) and a0042F15 (51.3 cM) showed the greatest recombination frequency (0.68 PF/cM) within the pericentromeric region of rice chromosome 5 (Fig. 5, Table 2), suggesting that although recombination in general is suppressed within the pericentromeric region, it is not evenly distributed within the region.

Increasing recombination and average chromosomal condensation at the short arm of rice chromosome 5

The average physical distance between markers in the region delineated by P0036D10 (0–3 cM, Fig. 2a–c) and P0033D06 (19 cM, Fig. 2b) was calculated as 0.36 PF/cM (Tables 1, 2), which was almost a threefold greater recombination frequency than average. The average physical distance between recombination events in the region spanned by P0033D06 (19.0 cM, Fig. 2b) and OJ1725E07 (36.4 cM, Fig. 2c) was calculated as

0.80 PF/cM (Fig. 5, Tables 1, 2). These results suggested that the recombination was increasing toward the distal end of the short arm, but no significant differences were found at the remainder of the regions at the short arm (Table 2). Clone OJ1725E07 (36.4 cM, Fig. 2c) was adjacent to the most highly condensed region (38.3–47.2 cM) on rice chromosome 5. The pachytene chromosome 5 showed less condensation with a PF/CF = 0.78, within the interval between the positions of the FISH signal assigned by OJ1725E07 (36.4 cM, Fig. 2c) and the distal end of the short arm (Fig. 5, Tables 1, 2), but showed an average condensation with a PF/CF = 1.00 at its telomeric region (Table 2).

Average recombination frequency and less chromatin condensation at the long arm of rice chromosome 5

Cytological observations suggested that the long arm of rice chromosome 5, except for the proximal region, showed less condensation at the pachytene stage (Fig. 1). The DAPI stained pattern of the long arm of chromosome 5 at the pachytene stage displayed an uniform staining intensity, although it had a few faint intervals (Fig. 1). Except for the pericentromeric region (54.6–60.7 cM), the chromatin of the long arm of rice chromosome 5 and the interval from the distal end of the short arm to 36.4 cM showed a similar DAPI-staining characteristics, with the same average PF/CF value as 0.78 (Table 2). However, the former had lower recombination frequency (0.89 PF/cM) than the latter (0.57 PF/cM).

The condensation degree of chromatin of the intervals separated by OJ1058F05 (87.4 cM, Fig. 2d) and OJ1123F01 (103.9 cM, Fig. 3d) was calculated as 1.26 PF/CF, which was closest to the degree of condensation of the pericentromeric region (1.65 PF/CF) on the long arm of rice chromosome 5 (Fig. 5, Tables 1, 2). This was followed by the chromatin at the telomeric region from OJ1345B12 (110.7 cM, Fig. 2d) to a0035I01 (122.3 cM, Fig. 2a, b), which showed a degree of condensation of 0.90 PF/CF. The remainder of the segments at the long arm of rice chromosome 5 were less condensed. The interval separated by OJ1058F05 (87.4 cM, Fig. 2d) and a0006J12 (75 cM, Figs. 2a, 3d) was the least compressed segment on rice chromosome 5, having a ratio of 0.52 PF/CF (Fig. 5, Tables 1, 2). Next were the intervals between a0077J17 (60.7 cM, Figs. 2f, 3d, e) and a0009L15 (64.1 cM, Fig. 3f) and between OJ1123F01 (103.9 cM, Fig. 3d) and OJ1345B12 (110.7 cM, Fig. 2b), both of which had a ratio of 0.60 PF/CF (Table 2).

The average physical size per centiMorgan on the long arm (Fig. 5, Table 2), in an interstitial segment between a0077J17 (60.7 cM) and a0009L15 (64.1 cM), was 0.66 PF/cM, which was the smallest value on the long arm of rice chromosome 5 (Fig. 5, Tables 1, 2); while recombination frequency seemed not increasing toward the end of the long arm (Table 2).

The resolution of BAC–FISH on rice pachytene chromosomes

In this study, high-resolution BAC–FISH on rice pachytene chromosomes is presented. We were able to resolve two BAC clones, spaced less than 100 kb, on pachytene chromosomes. The physical distances between P0499F10 and a0042F15 at 49.4–51.3 cM (Fig. 2e) and a0077J17 and OJ1320D10 at 60.7–62.7 cM (Fig. 2f), which were clone pairs flanking physical gaps generated by genomic sequencing, could be resolved on pachytene chromosomes using dual-colored FISH experiments. These two gaps were later filled by the screening of a 40-kb-insert rice fosmid library and PCR screening method (Cheng et al. 2005).

Discussion

Features of rice chromosome 5

We developed a cytogenetic map showing integration of information from the genetic and physical maps of rice chromosome 5. FISH mapping revealed that almost the entire length of rice chromosome 5 had been covered by the physical map constructed by Cheng et al. (2005) as shown in Fig. 2a, b. In addition, the precise position of the genetic centromere on rice chromosome 5 was assigned by FISH using probe pRCS2, which contains the CentO satellite that marks the functional rice centromeres (Cheng et al. 2002).

Both the gene density and recombination events, increasing toward the distal region of the chromosome arms, but reducing toward the pericentromeric region, have been reported in several plant species, especially species with large genomes, such as tomato, wheat, and barley (Tanksley et al. 1992; Delaney et al. 1995a, b; Sherman and Stack 1995; Kunzel et al. 2000). In silico comparison of the genetic and physical maps of rice revealed that recombination was severely suppressed at the centromeric regions, as well as on the short arms of chromosomes 4 and 10 (Chen et al. 2002), which were composed of mainly heterochromatin. These analyses were similar to the FISH analysis on chromosome 10, reported by Cheng et al. (2001b) and on chromosome 4 of *A. thaliana* (Fransz et al. 2000). The discordance of distances between the markers on different maps was also reported on rice chromosome 9 (Kato et al. 2003). In the present study, we found that the severe suppression on recombination was restricted to a limited region, spanned by the genetic centromere; however, increasing recombination was only found at the distal segment of the short arm of rice chromosome 5.

Our results revealed uneven distribution of the recombination events and the degree of chromatin condensation along the entire length of chromosome 5. Detailed comparisons revealed that the recombination events were distributed unevenly and that chromatin condensed at various degrees along the entire length of

chromosome 5 (Table 2, Fig. 5). The highly condensed regions were not necessarily low recombination regions; however; for example, the average physical distance between recombination events was similar in the most condensed interval spanned by b1007D10 (38.3 cM) and b0012L23 (47.2 cM) and in the loosest interval spanned by b0006J12 (75 cM) and OJ1058F05 (87.4 cM), calculated as 0.80 PF/cM and 0.72 PF/cM, respectively (Table 2). Severe suppression of recombination events was found in the genetic centromere region on the genetic map, spanned by a0018H09 (54.3–54.6 cM) and b1023E01 (54.6–55.4 cM), which had scarcely any recombination events, although showing a similar condensation degree to the interval between OJ1058F05 (87.4 cM) and OJ1123F01 (103.9 cM) at the long arm of chromosome 5 (Table 2). It suggests that rice chromosome 5 may be less compressed than the average compressed ratio of rice chromosomes. The compressed ratio of rice chromosome 5 at pachytene stage was estimated as 30.02 Mb/46.49 μm = 646 kb/μm, while the average ratio of rice chromosomes at pachytene stage (in total length 462 μm, according to Cheng et al. 2001a) was estimated as 430 Mb/462 μm = 930 kb/μm.

The segment at the north of P0033D06 (19 cM) on the short arm, including the telomere, showed the greatest recombination frequency (0.36 PF/cM, Table 2) on rice chromosome 5. However, although the chromatin was less condensed (Fig. 5, Table 2) being mainly euchromatin (Fig. 2), recombination did not significantly increase at the long arm of rice chromosome 5 (Fig. 5, Table 2). This suggests that less RFLP markers were available on the long arm of rice chromosome 5. Clones b0012L23 (47.2 cM), a0009L15 (64.1 cM), b0042J17 (87.4 cM), and OJ1345B12 (110.7 cM) were located at the northern clone flanked physical gaps 1, 2, 3, and 4, respectively, of the rice chromosome 5 pseudomolecule (Cheng et al. 2005). FISH results assigned the cytological position of gap 1 (47.2 cM) to the highly condensed interstitial region of the short arm of rice chromosome 5, and the position of gap 2 (64.1 cM) near the highly condensed proximal region on the long arm of rice chromosome 5. Highly condensed chromatin is known to be composed of mostly repetitive sequences (for review, see Fransz et al. 2003), which may make the sequencing process difficult. Gap 3 (87.4 cM) was localized at the loosest region on rice chromosome 5 with an average recombination frequency (Table 2), while gap 4 (110.7 cM) was localized at the region with the least recombination frequency on the long arm of chromosome 5. It suggests that there may be not enough markers available for screening BACs/PACs as bridge clones to fill these gaps on the pseudomolecule of rice chromosome 5.

Chromosome 5 homologous sequences in whole rice genome

In this study, FISH experiments were successfully carried out yielding strong primary signals on chromosome 5 from most BACs/PACs, although low levels of dispersed signals derived from some clones were observed. It was not surprising to find that the BAC probes sometimes hybridized to more than one site on the rice chromosomes complement. In general, most noticeable signals were found on chromosome 5 in all the FISH experiments performed in this study. Since we did not apply unlabeled genomic DNA for blocking in the hybridization mix, these weak signals were presumed to result from hybridization of the probe fragments to any other region with substantial homology to the sequence of the probe or from the dispersed repeats within these BAC inserts. For example, a0035I01 (122.3 cM) at the distal end of the long arm of chromosome 5 has been known to contain blocks of the Os 48 repeat family (Cheng et al. 2005). Os 48 is an AA genome-specific tandem repetitive sequence, organized as long arrays of 355 bp repetitive units (Wu and Wu 1987). Os 48 repeats have been ISH/FISH-mapped on specific rice chromosomal ends, including the long arm end of chromosome 5 (Wu et al. 1990; Cheng et al. 2001c). Besides, the order of individual BAC/PAC–FISH loci, mapped along the entire length of chromosome 5 and presented in this study, was fully concordant to that of the marker loci along the genetic linkage map.

Those two clones, a0018H09 and b1023E01, flanking the centromere of chromosome 5 were also detected on several chromosomes by FISH in this study (Fig. 4). It suggests that these two clones may contain repetitive sequences clustering at the pericentromeric regions of several chromosomes, or contain duplicated segments within rice genome. Chromosomal duplications occur widely in all organisms, which are believed to be important in genome evolution (Lupski et al. 1996). Chromosomal segments were found duplicated in distal ends of the short arms of rice chromosomes 11 and 12 (Wu et al. 1998), in chromosome 1 and chromosome 5 (Salse et al. 2004), even in whole genome (Yu et al. 2005) by intensively in silico analyzed the genomic structure and composition through the effective physical mapping of DNA markers in rice genome. However, such studies need a large number of DNA markers and a well-established genome sequencing data. It is expensive to develop a whole genome physical map by BAC contig assembly for plant species with very large genomes. Besides, genetic maps with dense RFLP markers may be not available for those interested plant species. Pachytene chromosome-based FISH method provides an alternative and useful solution to investigate the chromosome duplication events in the rice genome, as has been performed in human. Abundant paralogous relationships among sites dispersed across the human chromosomes have been found by FISH analysis (The BAC Resource Consortium 2001).

Perspectives of integrated cytogenetic map of rice chromosomes

A detailed comparison of the genetic and cytological maps is essential in revealing interesting cytogenetic

properties, such as chromosomal behavior in meiosis and the relationship between the sequence data and the chromosomal organization–function relationship (Lam et al. 2004). Information provided by an integrated cytogenetic map will (1) facilitate the selection of clones for further annotation and study; (2) be helpful for the placement of unanchored BACs/PACs onto rice chromosomes; and (3) be useful for investigation of homologous sequence distribution in relative genomes for synteny research (Harper and Cande 2000).

The integrated map presented in this study demonstrates the distribution of recombination and the degree of chromosomal condensation of rice chromosome 5. It indicates the positions of BAC/PAC clones on the pachytene chromosome, related to the cytological features of chromosome and recombination events distribution, providing useful information for the evaluation and selection of clones before further annotation and study. BAC–FISH on pachytene chromosomes allows the placement of those unanchored BAC clones in the integrated cytogenetic map, and then assigns their positions on the genetic map, based on their cytological position, relative to previously localized and genetically mapped FISH probes. The high-resolution integrated cytogenetic map of rice chromosome 5 has provided a fundamental system for such an approach. Except for that two clones, a0018H09 and b1023E01, flanking the centromere region (Fig. 4) and the clone a0035I01 at the distal end of the long arm (Fig. 2a, b), fewer paralogous sites were found within the whole rice genome based on FISH mapping with the rest of BACs or PACs in this study. This suggests that these RFLP marker anchored BACs/PACs are suitable to investigate genomic organization among *Oryza* species at the chromosomal level.

High-resolution cytogenetic maps would be useful in the comparative genomics and map-based gene isolation. Cytogenetic maps have been useful in the comparison of synteny between relative genomes, especially for complex-genome organisms, with large amounts of repetitive DNA, such as maize and wheat. A universal molecular cytogenetic mapping system would facilitate both integration of genetic information among grasses, and direct genetic and cytogenetic studies of chromosome organization and evolution. The integrated molecular cytogenetic map created in this study underpins the beginning of chromosome organizational comparisons among *Oryza* species and other cereal genomes.

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