A physical map of rice chromosome 5 was constructed with yeast artificial chromosome (YAC) clones along a high-resolution molecular linkage map carrying 118 DNA markers distributed over 123.7 cM of genomic DNA. YAC clones have been identified by colony and Southern hybridization for 105 restriction fragment length polymorphism (RFLP) markers and by polymerase chain reaction (PCR) screening for 8 sequence-tagged site (STS) markers and 5 randomly amplified polymorphic DNA (RAPD) markers. Of 458 YACs, 235 individual YACs with an average insert length of 350 kb were selected and ordered on chromosome 5 from the YAC library. Forty-eight contigs covering nearly 21 Mb were formed on the chromosome 5; the longest one was 6 cM and covered 1.5 Mb. The length covered with YAC clones corresponded to 62% of the total length of chromosome 5. There were many multicopy sequences of expressed genes on chromosome 5. The distribution of many copies of these expressed gene sequences was determined by YAC Southern hybridization and is discussed. A physical map with these characteristics provides a powerful tool for elucidation of genome structure and extraction of useful genetic information in rice.

Key words: physical mapping; YAC contig; rice genome; rice chromosome 5

1. Introduction

Rice is the most important food crop in the world and genetic analysis of its phenotype markers has been carried out for many years.1,2 Genome analysis of rice based on these genetic linkage data made possible the construction of two major rice restriction fragment length polymorphism (RFLP) linkage maps with about 6503 and nearly 1400 DNA markers.4 The highest resolution genetic map contains 1383 DNA markers at average intervals of 1.1 cM.4 Meanwhile, a rice yeast artificial chromosome (YAC) library which consists of approximately 7000 YAC clones with an average insert length of 350 kb was prepared first for the construction of a physical map.5 Construction of a physical map may be the best way to unravel the genome structure in rice and to isolate and manipulate useful genes of agronomical traits. It is also quite useful to construct a physical map which covers the whole rice genome with YAC contigs and to compare the physical map together with a genetic map. Since the genome of rice is 430 Mb long and since 1383 DNA markers have been precisely mapped, the average interval between these markers is 300 kb. Since our YAC library is composed of clones with an average insert size of 350 kb, we expected that aligning the YAC clones on all of these DNA markers would show that most chromosome regions of the rice genome could be covered with YAC contigs.

Construction of physical maps by arranging YAC contigs has been done for Arabidopsis, a model of higher plants, and for humans. The physical map of Arabidopsis chromosome 4, which consists of 563 YAC clones covering more than 90% of the chromosome and an estimated 82.5 cM, has been constructed to analyze the organization of the chromosome.6 Those YAC clones were selected using a total of 263 probes and the total size of YAC contigs has reached approximately 17 Mb. The resulting YAC contig map for Arabidopsis chromosome 5 consists of 35 contigs totaling about 21 Mb.7 We have already published a physical map of rice chromosome 6,8 60% of which is covered with YAC clones and have constructed YAC contigs for chromosome 1, 8, 9, 10 and 12. Here we describe the construction and characterization of a physical map of rice chromosome 5. Physical maps of the remaining rice chromosomes will appear in our continuing series of reports.

2. Materials and Methods

2.1. YAC library and DNA markers

To construct a physical map of the rice genome, a YAC library consisting of 6934 clones with an average
insert length of 350 kb was prepared from cultured cells of *Oryza sativa L. cv. Nipponbare*. The YAC library is estimated to cover six rice genome equivalents. A set of 118 DNA markers, consisting of 79 cDNA markers, 9 genomic DNA markers, 5 Not I-linking clones, 7 wheat DNA markers, 4 YAC end-clones, 1 telomere-associated clone, 8 STS markers and 5 randomly amplified polymorphic DNA (RAPD) markers, has been located on chromosome 5 at an average interval of 1 cM. This high density linkage map was constructed with an F_2 population from parent lines of Nipponbare (*O. japonica* rice) and Kasalath (*O. indica* rice).

2.2. Screening of the YAC library and YAC contig assembly

Two methods were adopted for YAC screening: the colony-hybridization method with ECL (Amersham)-labeled RFLP markers and the three-dimensional polymerase chain reaction (PCR) screening method with sequence-tagged sites (STS) and RAPD markers. For the first method, five sheets of high density replica filter dotted with 6934 YAC clones were used for colony-hybridization as described previously. After a labeled RFLP marker was colony-hybridized with these filters, DNAs from positive YAC clones were extracted and prepared for Southern blot analysis. YACs which contained the mapped DNA fragments on chromosome 5 were selected and placed in the corresponding positions to make YAC contigs. The sizes of the YAC clones comprising the array of the minimal tiling path on the physical map of chromosome 5 were scaled by Southern hybridization with a YAC vector probe following clamped homogeneous electric field (CHEF) gel fractionation.

3. Results and Discussion

3.1. YAC screening and positioning

One hundred and eighty markers on chromosome 5 identified 458 YAC clones from a total of 3123 colony hybridized YACs by Southern hybridization and PCR confirmation of individual YACs. Of these 458 individual YACs, 235 were assigned to chromosome 5 and 41 appeared to be located on other chromosomes: 7 on chromosome 1, 8 on chromosome 4, 5 on chromosome 6, 11 on chromosome 7 and 10 on chromosome 9. Moreover, unmapped loci of multicopy DNA marker sequences were also localized in 182 YACs by Southern analysis of all candidate YACs. Many copies of these multicopy DNA marker sequences were distributed on chromosome 5 as well as on other chromosomes. An ordered map of YAC clones on rice chromosome 5 (Fig. 1) was obtained by YAC landing with 111 DNA probes out of the 118 chromosome 5 markers on the high density molecular map. Seven DNA markers could not be used in YAC selection as probes, because the DNA fragments of their mapped Nipponbare bands could not be distinguished from yeast DNA bands. In addition to these 7 DNA markers, 11 markers failed to identify any YAC clones from our YAC library.

On the genetic map, we could not order many DNA markers which were located together on single map positions. Alignment of multiple YAC clones on these DNA markers resolved the positions of many clustered markers, as, for example, in region 1 from R2558 to C288A, and in region 2, from C1264 to C999B (see Fig. 1). Fifty chimeric YACs (labelled with the letter “C” after the YAC clone names in Fig. 1) were found through the detection of the same YAC clones assigned to other chromosomes. On average 3.9 YACs were selected by individual markers; at most 20 YACs were obtained by screening with a single marker, marker C1255 located in region 1. Only 24 out of 100 RFLP markers on chromosome 5 were single-copy sequences in the genome; in other words, 24 DNA markers were mapped to only single positions on chromosome 5. Southern analysis with genomic DNAs of the other 76 RFLP markers revealed multiple hybridization patterns. Thus compared with other rice chromosomes, chromosome 5 has many multicopy DNA markers. Most of these multicopy DNA markers were derived from expressed genes and 35 markers show homology to known genes. These expressed sequences are available with their accession numbers in the previously published RFLP map.

The high-density rice RFLP map was constructed by use of the distinct polymorphism between Nipponbare and Kasalath. However, there were 13 dominant DNA markers which were detectable only in Kasalath genomic DNA but not in Nipponbare DNA. The high-density rice RFLP map was constructed by use of the distinct polymorphism between Nipponbare and Kasalath. However, there were 13 dominant DNA markers which were detectable only in Kasalath genomic DNA but not in Nipponbare DNA.

Figure 1. Chromosome 5 is divided into 2 regions from the upper to the lower ends. The genetic map of chromosome 5 is shown on the right side and the physical map, arranged with YAC clones, is aligned on the left side. Most markers are shown in the middle of each region to correspond to identified YACs. Markers with which YACs could not be selected from the YAC library are shown on the right side of the genetic map and markers which could not used for screening are underlined. On the genetic map, the gray bar represents the full length of chromosome 5 in centimorgans and the black bars show the regions connected with YAC clones as contigs. On the physical map, bars containing circles and/or squares represent the YAC clones. Circles show each DNA marker hybridized to the YAC clone and black squares indicate the end clone of each YAC. Y number represents the name of each YAC clone. Chimeric YACs are indicated by letter “C” following the Y number. White squares show co-existing DNA bands linked with the mapped bands on the same position. Black bars carrying black circles and/or squares represent a set of YAC clones comprising minimal overlaps for chromosome 5.
Figure 1. A physical map of rice chromosome 5.
for allelic bands, because of ambiguous Nipponbare banding in Southern analysis. For example, DNA marker C903 developed a smear hybridization pattern with Nipponbare genomic DNA (Fig. 2A) and it was quite difficult to distinguish genomic fragments as an allele for a 3.9-kb Kasalath band located on chromosome 5. The eight positive Nipponbare YACs were, however, found by Southern hybridization analysis to have either 23 kb or 15 kb Nipponbare fragments (Fig. 2B). Four YACs selected with DNA markers C128 and R3182, which were linked to C903 at 0 cM distance, had 23 kb bands. These markers and YACs could be arranged with good consistency (Fig. 2C). Thus even a Kasalath-dominant marker could seldom identify YAC clones possessing a fragment of the corresponding Nipponbare allele.

3.2. YAC contig assembly and scaling

There were 20 YAC contigs spanning more than 2 different marker locations and their combined length amounted to 27.4 cM (shown by thick lines in Fig. 1).
Figure 3. Different DNA copies assigned on different chromosomes. A. Hybridization pattern in YAC Southern analysis digested with HindIII and probed with a multicopy DNA marker, C67. The 3.2-kb fragment was mapped on chromosome 5 as the C67B locus and the 3.8- and 2.4-kb fragments were mapped on chromosome 7 as the C67A locus in a previous study.\textsuperscript{4} A YAC clone, Y4170 indicated by *, which carried the 3.2-kb band, was located on a locus of chromosome 5 as shown in B. B and C. YAC arrangements on the positions of C67 DNA marker loci on chromosomes 5 and 7, respectively.

The longest contig that was formed by 3 distinct YACs covers 6 cM from R372 to C1239 in region 1. Marker-dense zones where multiple DNA markers were located at the same positions were observed in the lower parts of regions 1 and 2 (see Fig. 1). YACs arrayed on these markers formed contigs of about 3 and 2 Mb in length, respectively. The two single YAC clones covering regions longer than 4 cM were Y5604 for 4.2 cM in the lower part of region 1 and Y3804 for 4.0 cM in the upper part of region 2. The 20 YAC contigs were estimated to cover 7.5 Mb in combined length (273 kb/cM x 27.4 cM; 1 cM corresponds to 273 kb, since the genome size is 430 Mb and the map length is 1575 cM).

YAC islands which carried more than 2 YACs but were assigned to single marker positions were found for 21 marker positions on the map. These 21 YAC islands were calculated to cover 11.0 Mb, because multiple YACs for one position can be assumed to overlap each other by 50% in length (350 kb x 1.5 x 21). Seven DNA marker Probes selected only 1 YAC clone. As our YAC clones have an average insert size of 350 kb, these 7 YACs are estimated to cover 2.45 Mb in total (350 kb x 7). Thus, a total of 21 Mb length (7.5 + 11.0 + 2.45 = 20.95 Mb) should be covered by the YAC clones we arrayed on chromosome 5. The coverage of chromosome 5 with these YACs amounts to 62% (21 Mb/34 Mb x 100) of the chromosome's total length, because the total length of chromosome 5 was assumed to be 123.7 cM or 33.77 Mb (273 kb/cM x 123.7 cM).

There was a zone of about 10 cM between R3103 and
L1013 in region 2 that no YAC clone from our YAC library could identify (see Fig. 1). This region may contain DNA sequences which are difficult to clone into YACs. Moreover, there were several other regions of more than 5 cM length (gap regions) on which no YACs were located. To elucidate the physical characteristics of these genetic gap regions, it would be necessary to clone the DNAs into BAC and cosmid libraries. Chromosome walking and fingerprinting will also help to fill in the gap regions between contigs.

3.3. Distribution of gene copies on the physical map

Seventy-six out of 100 RFLP markers on chromosome 5 were multicyclic sequences. Six out of these 76 markers could identify YACs carrying sequences located on both chromosome 5 and other chromosomes. The other 70 RFLP markers detected not only YACs which had sequences located on chromosome 5, but also YACs carrying sequences which have not yet mapped on the linkage map by Southern hybridization.

cDNA clone C67, isolated from rice callus, is one of these repetitive sequence DNA markers. This clone exhibited seven hybridized bands in Nipponbare genomic DNA when digested with HindIII (Fig. 3A). In order of decreasing fragment size, these bands were designated C67-1 (6.5 kb), -2 (5.0 kb), -3 (4.9 kb), -4 (4.0 kb), -5 (3.8 kb), -6 (3.2 kb), -7 (2.4 kb) and -8 (1.4 kb). RFLP analysis was used map the C67-6 (3.2 kb) band onto chromosome 5 as the locus of C67B and the C67-5 (3.8 kb) and C67-7 (2.4 kb) bands onto chromosome 7 as the locus of C67A. The loci of the other bands, C67-1 (6.5 kb), -2 (5.0 kb), -3 (4.9 kb), -4 (4.0 kb) and -8 (1.4 kb), on the linkage map have not yet been determined, because of the lack of polymorphism between the parental rice strains of our mapped population. Sixteen YAC clones were identified by Southern hybridization with the C67 probe. Only one YAC clone, Y4170, carried the C67-6 (3.2 kb) fragment mapped on chromosome 5 (Fig. 3A and 3B). In some cases, YACs which were located on the physical map were found to carry unmapped DNA fragments of the marker sequence. Those YACs could be used to assign unmapped copies onto distinct positions of chromosome 5. Eleven YACs located on chromosome 7 include not only mapped fragments of C67-5 (3.8 kb) and C67-7 (2.4 kb), but also C67-2 (5.0 kb) and C67-4 (4.0 kb) fragments as unmapped loci, suggesting that these 5.0- and 4.0-kb bands of the C67 marker sequence are located very near to the 3.8- and 2.4-kb bands within the YAC on chromosome 7. Four other YACs carrying unmapped copy bands of C67-1, -3 and -8 were found to locate away from both these regions of chromosome 5 and 7. Thus more accurate YAC physical mapping could be conducted by investigating overlaps of YAC clones with multicyclic DNA sequences.

Our goal is the construction of a complete physical map, which is indispensable for resolving genome structure as well as isolating valuable genes. Additional efforts to facilitate rice physical map construction are mapping all YAC clones on the chromosomes by YAC landing with 900 more newly mapped DNA markers and by chromosome walking. Gaps which will still remain after all YAC arrangement will be filled with BAC/cosmid clones.

All materials and information for the high density YAC filters, YAC clones and DNA markers used in this study are available upon request from the MAFF DNA bank via the World Wide Web at http://bank.dna.affrc.go.jp.

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