Physical Mapping of Rice Chromosome 1 with Yeast Artificial Chromosomes (YACs)

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Abstract

We have constructed a physical map of rice chromosome 1 using yeast artificial chromosomes (YACs). A YAC library of 350 kb average insert size, covering about 6 rice haploid genome equivalents, was screened using 182 DNA markers which we had previously located on chromosome 1, by colony hybridization and polymerase chain reaction (PCR) amplification. One hundred and sixty-two DNA markers identified at least one YAC each carrying one, two or more marker sequences, for a total of 476 clones. Of these identified YACs, 284 were located in their original positions on chromosome 1. These 284 YACs defined 69 YAC contigs or islands which are estimated to cover more than 60% of the total chromosome length. The use of mapped DNA markers in constructing a physical map facilitates the integration of genetic and physical maps, as well as fine ordering of the DNA markers, especially at sites where the markers are clustered tightly on the genetic map. Our high density molecular map has been proven, by chromosome landing with YACs using mapped DNA markers, to cover more than half of the entire length of chromosome 1. The remaining 192 YACs were selected by other copies of DNA markers that mapped on chromosome 1. This description of the YAC contigs formed on chromosome 1 constitutes the second report of rice physical mapping, following that for chromosome 6.

Key words: Yeast artificial chromosome (YAC); physical mapping; rice

1. Introduction

A complete physical map of a genome is useful for studying its structure, function and evolution. Such a map also provides unique materials and information for positional cloning of genes for which the proteins and/or biochemical functions are unknown. Interest has focused on strategies for constructing physical maps of entire genomes in several genome research projects including those for humans, animals and higher plants.1-4

Rice is one of the most important staple crops in the world. Studies of its genetics and breeding have been conducted by many researchers, leading to the accumulation of a huge volume of information. In addition, rice has been a model plant for genome studies of cereals,5 because it has the smallest genome size (4.3 x 10^8 bp) among cereal crops, and because it has very good synteny with other cereals such as wheat7,8 and maize.9 Therefore, physical mapping of the rice genome will be very useful not only for rice research and breeding, but also for studies of other cereal plants.

Recent efforts in rice genome mapping with molecular markers have made available several high-density molecular linkage maps.10-12 In particular, the map reported previously12 contains 1383 DNA markers, which were distributed along the rice genome at intervals averaging 300 kb, and covering the genome almost evenly. Also, a rice YAC library, which contains 6932 clones with an average insert length of 350 kb and covers about 6 haploid genome equivalents, has been constructed.13 Characterization of this YAC library showed it to be suitable for physical mapping of the rice genome. The high-density markers on the restriction fragment length polymorphism (RFLP) linkage map and the good quality of this YAC library make it possible to physically map the rice genome. To facilitate the use of rice as a model cereal in genome research, we have been generating a physical map of the rice genome with YACs using the 1383 DNA markers we had mapped. This paper reports the first compilation of YAC contigs for the physical mapping of rice chromosome 1, and also discusses our strategy for completion of the physical map of the entire rice genome.
2. Materials and Methods

2.1. YAC library
The rice YAC library constructed previously was used to land these clones on their original positions on the chromosome. The YAC library contains 6932 clones with an average insert length of 350 kb. These YACs were stored individually in wells of 96-well microtiter plates, and dotted onto 5 membrane filters for screening by colony hybridization, or subpooled for screening by polymerase chain reaction (PCR) amplification, as described earlier.

2.2. DNA probe
Previously, 193 DNA markers, including 37 genomic clones, 133 cDNA clones, 4 randomly amplified polymorphic DNA (RAPD) markers, 10 sequence-tagged sites (STS) markers, and 9 wheat clones, were mapped on rice chromosome 1. Of these, 182 markers were used as probes for screening the YAC library. The other 11 markers could not be utilized in this study for various reasons. The RFLP marker fragments cloned in pBlueScript II SK* (TOYOBO) were amplified by PCR with M13 primers; M4 and RV, purchased from Takara Shuzo and labeled with peroxidase using the ECL direct DNA labeling kit (Amersham) to make probes for colony hybridization. Oligonucleotide primers of the STS markers were synthesized and used for PCR amplification screening.

2.3. Screening the YAC library with mapped DNA markers
YAC screening by RFLP markers was carried out by colony hybridization experiments. The procedure was performed as described with a few minor modifications. Clones selected by colony hybridization were confirmed by Southern (DNA) blot analysis of every candidate YAC. DNA isolation, restriction enzyme digestion and electrophoresis of candidate YACs were conducted as previously described. DNAs of rice cv. Nipponbare and yeast strain AB1380 were used as controls in Southern analysis experiments. Hybridization and signal detection were done using the ECL system according to the protocol provided by the manufacturer (Amersham).

Screening by STS markers was performed through PCR amplification using a 3-step PCR screening system. First, PCR amplification was done with eight pools of YACs; each pool from nine 96-well microtiter plates stock. Secondly, PCR was done with each X pool of 108 YACs, Y pool of 72 YACs and Z pool of 96 YACs. All candidates were individually confirmed by amplifying with the STS primers in the final step. Nipponbare and yeast host strain AB1380 were also used as controls in the PCR amplification.

2.4. Assignment of YACs and construction of a physical map
The chromosomal locations of YACs were confirmed by Southern analysis through comparisons of the fragments which appeared in each YAC with those of genetically mapped RFLP markers or PCR products of STS markers. Hybridization of different YACs to one or more linked DNA markers established overlaps and extended the physical map. From these analysis, YACs were assigned to their positions along the genetic linkage map of rice chromosome 1 (Fig. 1).

2.5. Examination of YAC insert length
To examine the insert length of YACs, high-molecular-weight DNAs of YACs were prepared in agarose plugs. Pulsed field gel electrophoresis (PFGE) and blotting of DNA were followed by hybridization with pYAC4 left arm-specific sequence and subsequent detection of YAC length.

3. Results

3.1. Selection and landing of YACs on chromosome 1
Out of 182 markers genetically mapped on rice chromosome 1, 162 DNA markers could detect each at least one positive YAC clone. A total of 476 positive YACs were selected from the library through colony hybridization together with Southern analysis or PCR amplification. The remaining 20 markers, including 12 cDNA clones, 5 genomic clones, 2 STS markers, and 1 wheat clone, failed to identify any YAC by repeated colony hybridization and PCR amplification, indicating that the library does not contain the DNA fragments from which these markers derived.

The chromosomal locations of the 476 YACs were determined by Southern and PCR analyses of the mapped DNA fragments. Among these, 284 YAC clones which carry 162 DNA marker sequences were assigned to chromosome 1 (Fig. 1). Thirty other YACs were located on chromosomes 2 (8 YACs), 3 (2 YACs), 4 (5 YACs), 5 (12 YACs), and 11 (3 YACs), as some multi-copy markers which mapped on multiple loci on different chromosomes, including chromosome 1. The chromosomal locations of the other 162 YACs have not yet been detected, because the restriction fragment sites carried on these YACs have not yet been mapped.

Of the 284 clones assigned to chromosome 1, 60 YACs constituting 21% of the total number, were found to be chimeric, because they also carried marker sequences mapped on other chromosomes or far-separated regions. These chimeric YACs and the deletion clones detected so far are marked in Fig. 1.

The physical map of chromosome 1 we constructed (Fig. 1) contains 69 YAC overlaps or islands. Twenty-
Figure 1. Alignment of YACs along the genetic linkage map of chromosome 1.
The linkage map is shown in five continuous regions from 1, the upper end, to 5, the lower end, as long vertical bars on the right side. YAC clones were arranged in accordance with the positions of DNA markers with which they hybridized. The names of DNA markers arranged between the linkage map and the YAC clones are those with which positive YACs were obtained. Markers listed at the right side of the linkage map are those with which no positive YACs were obtained. Solid lines on the linkage map correspond to regions in which the markers were spanned by YACs. Circles represent DNA markers located on each YAC. YACs carrying black circles represent clones comprising tentative minimum overlap clones on the chromosome and are given with their sequence lengths. Gray circles show that the YACs were selected by co-existing bands other than the mapped RFLP bands of the markers. Gray bars in part of some YACs indicate the absence of the corresponding intervening marker sequences, suggesting deletions of these clones. Black rectangles show the ends of YAC. The numbers of chimeric YACs are printed in white letters with black outlines.

Figure 1. Continued.
two YAC contigs cover a total of 45.7 cM with 63 positions containing 100 DNA markers along the linkage map. The longest contig is that of R886-C1456, which is 7.5 cM in length and spans 10 markers in region 3 (Fig. 1). Twenty-six single genetic positions harboring multiple DNA markers also picked up multiple YACs, forming contigs at each of these positions. The other 21 positions could identify only one YAC with each DNA marker.

Out of the 284 YACs assigned to chromosome 1, the insert lengths of 91 YACs were determined. These YACs consist of tentative minimum overlapping clones of the physical map. It was revealed that the minimum overlapping clones in the present physical map have a total coverage length of 31–37 Mb (Fig. 1).

3.2. Fine mapping of DNA markers using ordered YACs

There are 27 marker loci having two or more markers mapped to the same position in the genetic map (Fig. 1). The order of these markers has not been clarified by genetic mapping, because of the resolution limit imposed by using 186 F2 plants. Using YAC overlaps in several contigs, the orders of some of these DNA markers could be resolved. Individual DNA markers clustered on a position hybridized one, two or more YACs in the contig and, as a result, almost all such markers could be ordered by logical arrangement of YACs. For example, in the middle part of region 1, there are seven markers (R2018, L1082, C346, R2414, G2200, R2657A, and W170A) which were all mapped to the same position at 30.4 cM. The order as well as the orientation of these seven markers were deduced from the alignment of multiple YACs assigned with these markers, as shown in Fig. 1 (region 1). Y4780 hybridized to DNA markers of R2018, L1082, C346, R2414, and also to R1545 on the distal position next to R2018, and Y4261 hybridized to G2200, R2657A, W170A, and also to R2625 on the proximal position next to W170A, indicating that R2018, L1082, C346, and R2414 form a centromeric array of markers on this position.

4. Discussion

From the total rice genetic distance of 1575 cM12 and rice genome size of $4.3 \times 10^8$ bp,9 we can estimate the average physical size of each centimorgan of the rice genome as 273 kb ($4.3 \times 10^8$ bp/1575 = 273 kb). This implies that the 45.7 cM covered by the 22 YAC contigs corresponds to 12.5 Mb (45.7 x 273 kb = 12.5 Mb). Another 26 overlaps having multiple YACs were estimated to cover 13.7 Mb in total length, assuming a 525 kb average length because the size of multiple YAC overlaps on a single position may vary from 350 kb to 700 kb (26 x 525 kb = 13.7 Mb). The remaining 21 single YACs on 21 individual markers would cover 7.4 Mb (21 x 350 kb = 7.4 Mb). Therefore, the 284 YACs assigned on chromosome 1 would cover a total of 33.6 Mb of the chromosome. Considering that the genetic distance of chromosome 1 is 191.8 cM,12 the size of chromosome 1 would be 52.4 Mb (273 kb x 191.8 = 52.4 Mb). Thus, the first physical map reported here would cover about 64% of chromosome 1 (33.6 Mb/52.4 Mb). The total length of the set of YACs comprising tentative minimum clone overlaps ranged from 31 Mb to 37 Mb (Fig. 1), spanning the estimated total length of YAC contigs and islands of 33.6 Mb.

The usefulness of YAC contig formation for trapping target genes in YAC clones is also being demonstrated in rice. By selecting some YACs on chromosome 1, Van Houten et al.18 have physically mapped a flower morphogenesis gene, the extra glume (eg) locus. These YACs will provide unique materials for map-based cloning of this gene.

The use of mapped DNA markers in constructing the physical map allowed the integration of the genetic and physical maps. In this study, most of the markers used were cDNA clones, some of which have high homology with known genes.12 Isolation of YACs by these cDNA clones would provide useful materials for studying these genes, especially for the distribution and organization of gene copies in the genome.

Screening the library with markers located fairly evenly on chromosome 1 has also led us to once again evaluate the quality of our YAC library. Of the markers used in this study, about 90% (162 of 182 markers) successfully isolated at least one corresponding YAC, indicating that about 90% of the rice genome has been cloned in our YAC library. Of the 284 clones assigned to chromosome 1, 60 were chimeric clones which were also mapped to other chromosomes or other regions. This ratio is about half of the frequency of chimeric clones (40%) that was previously estimated by genetic mapping of the end fragments of YAC clones.13

As has been mentioned by many researchers, rice has become a model plant in the genome research of cereal crops. Ordered YACs from rice are powerful tools not only for understanding the genome structure of rice itself, but also for exploring the genomes of other cereals. The usefulness of rice YACs and related information in searches for other crop genes has been well demonstrated in wheat19 and barley.20 Rice chromosome 1 has a collinear relationship to chromosome group 3 of wheat9 and to chromosomes 3 and 8 of maize.9

We intend to use more than 100 additional DNA markers now being located on the genetic map of chromosome 1 to complete the rice physical map. The use of these additional DNA markers will allow us to identify YACs which might fill the gaps between ordered YACs in the current physical map. After arranging as many clones as possible with all of our DNA markers, we will be able
to map most of the remaining YACs, which have not been picked up by any of the DNA markers among our 6932 clones, by using end/sub-clones on either the genetic or the physical maps. Chromosome walking will also be helpful in filling the gap regions. In addition, we are trying to apply a fingerprinting approach to assemble YAC clones for generating longer contigs. We expect that completion of physical mapping with all of our YACs will eventually cover around 90% of the rice genome.

5. Availability of YAC Clones and Marker Information

All clones and information of YACs and DNA markers used in this study are available from the MAFF DNA Bank operated by the Ministry of Agriculture, Forestry and Fisheries of Japan. Request forms and information about the markers (positions in the linkage map, Southern blot hybridization images of RFLP markers, DDBJ accession numbers of RFLP marker sequences, primer sequences of RAPD and STS markers, etc.), high density YAC replica filters, and YAC clones are available on the world wide web at: http://bank.dna.affrc.go.jp.

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