An Ordered Clone Bank for Chromosome I of Saccharomyces cerevisiae

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Chromosome I of Saccharomyces cerevisiae DC5p9 was dissected into segments with an average size of 14.0 kb and closed into λ phage vectors. The physical maps of the resultant clones, totaling 205.9 kb, were used to construct an ordered clone bank of this chromosome.

The budding yeast Saccharomyces cerevisiae is one of the simplest eukaryotic organisms, and it has been extensively used in genetic studies. To date, more than 750 loci have been genetically mapped in 16 linkage groups (12). The genome of S. cerevisiae is about 14,000 kb in length and consists of 16 chromosomal DNA molecules of various lengths (1, 14). Of the 16 chromosomes, chromosome I is both genetically and physically the smallest (2, 12).

To analyze the structural and functional characteristics of the genome of S. cerevisiae, we have constructed ordered clone banks for its five small chromosomes, i.e., chromosomes I (this work), III (23), VI (24), and V and VIII (18). These ordered clone banks are useful in precise physical mapping of genes and analysis of their neighboring regions. They can also be exploited for the cloning of genes related to those of other organisms and for the identification and localization of actively transcribed genes under a variety of growth conditions. As part of our initial attempt, we report here on the construction of an ordered clone bank and a detailed physical map for chromosome I.

We used S. cerevisiae DC5p9 and prepared a chromosome I-specific clone library. High-resolution restriction maps of individual clones were constructed with eight 6-base-recognizing restriction endonucleases, i.e., BamHI, BglII, EcoRI, HindIII, KpnI, PstI, PvuII, and XhoI, and were compared with one another by using a computer program (9, 23). When overlapping regions of neighboring clones were short and the linking of clones was not certain, “packed array hybridiza-

FIG. 1. Physical map of chromosome I and alignment of clones. The restriction map was constructed by aligning and averaging the data for 126 clones. Only selected clones are shown here. The S. cerevisiae genomic fragments are inserted into λ phage vector EMBL4, except for those whose names start with E. For the latter clones, λ DASH II (Stratagene Co.) was used as a vector. Restriction enzyme cleavage sites: B, BamHI; G, BglII; E, EcoRI; H, HindIII; K, KpnI; P, PstI; V, PvuII; X, XhoI. The individual cleavage sites are indicated by short vertical bars from top to bottom in this order. Horizontal bars below the restriction map show representative ordered clones, their lengths corresponding to the extent of the chromosomal segments contained in them. Thick horizontal bars above the restriction map indicate the size and location of the genes physically mapped in this study (see the text).

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tion" (9) was performed. A total of 126 clones were thus sorted into one large continuously aligned clone group as shown in Fig. 1. The cumulative physical length of this clone group was 205.9 kb. The physical length of chromosome I of strain DCSP5 was estimated experimentally as approximately 245 kb by orthogonal-field-alternation gel electrophoresis (1) with phage λ concatemers as molecular size markers (23). Therefore, the above-mentioned cumulative physical length of the final clone group corresponds to about 84% of the physical length estimated by orthogonal-field-alternation gel electrophoresis.

It is known that the telomeres of most S. cerevisiae chromosomes contain conserved elements, termed X and Y' sequences (3), and their distribution among chromosomes is different from one strain to another (25). In the case of strain DCSP5, used in this work, neither the X nor the Y' sequence was found to hybridize with chromosome I (23). Thus, it was not possible to identify clones by walking from the telomeres inward or to evaluate whether the telomeres of chromosome I were included in our ordered clone bank. Steensma et al. (17) previously mapped the PHO11 gene at 3.4 kb from the right telomere by using the YCF vector (19), identifying PHO11-containing fragments, and measuring their sizes. Our restriction map (Fig. 1) in the 185.9- to 201.7-kb region (4.2 to 20.0 kb from the right end) was indistinguishable from the one reported by these authors. It thus appears that our ordered clone bank contains the right telomere region. This implies that the bank lacks an approximately 40-kb-long stretch from the left end. Therefore, we tried to extend this clone group towards the left telomere of chromosome I by repeating packed array hybridization. For this purpose, we used membrane filters with the DNAs of 4,800 EcoRI clones prepared from the whole genomic DNA of S. cerevisiae (18). However, no clone with which the existing clone group could be extended outward was obtained, despite the fact that nine EcoRI clones did positively hybridize with the clones mapped at the left end.

The PHO11 gene was reported to be duplicated on chromosomes I and VIII (17). In support of this, the restriction map of chromosome I (Fig. 1) at 183.9 through 201.3 kb is almost identical to the restriction map of chromosome VIII at 552.0 through 575.9 kb (18), except for the presence of a Ty element in this region of chromosome VIII. By determining the nucleotide sequences of the boundaries of the duplicated regions on the two chromosomes, we may be able to obtain clues as to how this duplication happened.

Attempts at physical dissection of S. cerevisiae chromosome I have been performed previously by Kaback and associates (4, 8, 16, 17, 22) and by Diel and Pringle (6). When these reports are taken together, a physical map amounting to about 75% of chromosome I, containing the cleavage sites of restriction enzymes BamHI, EcoRI, HindIII, PstI, PvuII, and XhoI, is available (different enzymes were used in different reports by these authors, and, therefore, the physical map data are not available for all of these restriction enzymes). Except for a few differences, their map and ours are in good agreement.

In the latest version of the genetic map of S. cerevisiae (edition 10), 18 genes are registered on chromosome I (12). Of these, nucleotide sequence data for nine genes are included in the GenBank nucleotide sequence data base (release 71.0). Using these sequence data and additional information provided in the respective references (5, 7, 10, 11, 15, 15, 20, 21), we could locate seven of the nine genes. In addition, the position of the chromosome I centromere (CEN1) could be determined on the basis of the report by

![FIG. 2. Integration of the physical and genetic maps of chromosome I. The genetic map (thin bar) was adapted from the work of Mortimer et al. (12), and the physical map (thick bar) was adapted from the data shown in Fig. 1. The left end is at the top, and the centromere is indicated by a small open circle. Scales for both maps are indicated at the bottom (cM, centimorgans).](http://jb.asm.org/Downloaded from http://jb.asm.org)


