MOLECULAR AND CELLULAR BIOLOGY, Jan. 1987, p. 410–419
0270-7306/87/010410-10$02.00/0

Vol. 7, No. 1

Molecular Cloning of Chromosome I DNA from *Saccharomyces cerevisiae*: Isolation and Analysis of the *CEN1-ADE1-CDC15* Region

H. YDE STEENSMA,† JOAN C. CROWLEY,‡ AND DAVID B. KABACK*

Department of Microbiology, University of Medicine and Dentistry of New Jersey, Graduate School of Biomedical Sciences, New Jersey Medical School, Newark, New Jersey 07103

Received 11 August 1986/Accepted 10 October 1986

To continue the systematic examination of the physical and genetic organization of an entire *Saccharomyces cerevisiae* chromosome, the DNA from the *CEN1-ADE1-CDC15* region from chromosome I was isolated and characterized. Starting with the previously cloned *ADE1* gene (J. C. Crowley and D. B. Kaback, J. Bacteriol. 159:413–417, 1984), a series of recombinant λ bacteriophages containing 82 kilobases of contiguous DNA from chromosome I were obtained by overlap hybridization. The cloned sequences were mapped with restriction endonucleases and oriented with respect to the genetic map by determining the physical positions of the *CDC15* gene and the centromeric DNA (*CEN1*). The *CDC15* gene was located by isolating plasmids from a YCp50 *S. cerevisiae* genomic library that complemented the *cdc15-1* mutation. *S. cerevisiae* sequences from these plasmids were found to be represented among those already obtained by overlap hybridization. The *cdc15-1*-complementing plasmids all shared only one intact transcribed region that was shown to contain the bona fide *CDC15* gene by in vitro gene disruption and one-step replacement to delete the chromosomal copy of this gene. This deletion produced a recessive lethal phenotype that was also recessive to *cdc15-1*. *CEN1* was located by finding a sequence from the appropriate part of the cloned region that stabilized the inheritance of autonomously replicating *S. cerevisiae* plasmid vectors. Finally, RNA blot hybridization and electron microscopy of R-loop-containing DNA were used to map transcribed regions in the 23 kilobases of DNA that went from *CEN1* to *CDC15*. In addition to the transcribed regions corresponding to the *ADE1* and *CDC15* genes, this DNA contained five regions that gave rise to polyadenylated RNA, at least two regions complementary to 4S RNA species, and a Ty1 transposable element. Notably, a higher than average proportion of the DNA examined was transcribed into RNA.

Physical studies suggest that 5,000 to 6,000 genes are expressed under conditions of vegetative growth in the yeast *Saccharomyces cerevisiae* (11, 28). In contrast, far fewer genes have been characterized genetically. To increase the number of known genes and reduce this discrepancy, we attempted to saturate the genetic map of one yeast chromosome with nonnutritional temperature-sensitive (*ts*) lethal mutations (31). Nonnutritional *ts* mutants have been used extensively to define most of the essential genes in bacteriophages T4 and T7 (14, 39, 52) and many essential genes in *S. cerevisiae* (20, 36, 37), *Escherichia coli* (1), and several other organisms (39). As a study involving saturation mutagenesis of an entire eucaryotic chromosome had not been done previously, we reasoned that these mutations would define most of the essential genes located on this chromosome, enhance our knowledge of the functions essential for vegetative growth, and provide a basis for estimating the total number of essential genes in the *S. cerevisiae* genome (31).

For this study, we chose chromosome I, the smallest *S. cerevisiae* chromosome. This chromosome contains 100 centimorgans (cM) between its two most distally mapped genes (37) and is only ca. 260 kilobases (kb) long (8, 44). Since there is, on average, one transcribed region per 2.2 to 2.8 kb of DNA in the *S. cerevisiae* genome, we estimated that chromosome I contains approximately 100 genes (11). However, our initial study showed that 32 independently isolated lethal *ts* mutations fell into only three complementation groups, which corresponded to the previously known genes *CDC24*, *PYK1*, and *CDC15* (31). In addition, two other essential genes on chromosome I, *MAK16* and *LET1*, were not represented among the mutants. This meant that either the technique used was incapable of uncovering all the essential genes on chromosome I or this chromosome harbors only five essential genes. If chromosome I is representative of the entire genome and there are only five essential genes per 260 kb, the entire 14,000-kb *S. cerevisiae* genome (33) would contain only 300 genes essential for growth on rich medium. This number of genes is much lower than expected. Therefore, additional studies are needed to determine whether chromosome I contains any more essential genes.

In addition to the five known essential genes, there are nine other known nutritional or nonessential genes on chromosome I. Thus, this chromosome contains a total of only 14 loci that have been detected so far by classic genetic means (37). This number also represents only a small percentage of the 100 genes that are expected to be on this chromosome. The discrepancy between the number of transcription units and the number of genetically defined loci has been referred to as the gene number paradox (31) and has been observed in extensively studied genomic regions of other organisms (4). The reasons for the discrepancy on chromosome I might be the following. (i) This chromosome indeed harbors fewer genes than expected from its length of DNA, (ii) Many genes on chromosome I if physically or functionally duplicated elsewhere on the genome would not be uncovered easily by mutational analysis. (iii) Many genes on chromosome I

* Corresponding author.
† Permanent address: Department of Microbiology and Enzymology, Delft University of Technology, Delft, The Netherlands.
‡ Present address: Department of Neurosciences, UMDNJ, New Jersey Medical School, Newark, NJ 07103.

TABLE 1. *S. cerevisiae* strains

Strain	Relevant genotype[a]	Reference
Y185	*MATa ade2 his8 lys2 +* *MATα + + + his2*	32
X1221a-7C[b]	*MATa ade1 leu1 trp1 arg4* *MATα 0 + + +*	7
DK8[c]	*MATa ade1 leu1 trp1 arg4* *MATα ade1 + + +*	29
X3402-15C[d]	*MATa ADE1 ura3 leu1 arg4* *ADE1*	35
DK329-4D	*MATα cdc15-1 ade1-1 ura3-1 trp1-1 his3-11,15 leu 2-3,112*	This study
DK358	*MATa cdc15-1 ade1-1 his3-11,15 leu2-3,112 +* *MATα + + his3-11,15 leu2-3,112 can1-100*	This study
DK358Ω1[e]	*cdc15-1* *cdc15::LEU2*	This study
DK357-3A	*MATa ade1-1 trp1-1 his3-11,15 leu2-3,112 gal10*	This study

[a] Some strains may carry additional nutritional markers.
[b] Monosomic for chromosome 1.
[c] Diploid related to X1221a-7C.
[d] Disomic for chromosome I.
[e] Same genotype as DK358 with the noted gene disruption in *CDC15*.

encode as yet unknown functions. (iv) Many genes on chromosome I encode functions that are nonessential in the laboratory.

To analyze the relative contributions of these factors to the gene number paradox and to determine whether there are more essential genes on chromosome I, we began a study whose goals were to clone all the DNA from chromosome I on recombinant DNA molecules, to localize transcribed sequences, to identify the physical locations of already known genes, to determine which genes are duplicated, and to determine which genes are essential for growth on rich medium. We recently reported the isolation and the location of transcribed regions on the 18 kb of chromosome I DNA surrounding the *CDC24* and *PYK1* genes (11). Here we report the isolation and analysis of the centromeric region from this chromosome.

We isolated a total of ca. 82 kb of chromosome I DNA from the region surrounding the previously isolated *ADE1* gene (12). This DNA was used for the localization of *CEN1* DNA and the *CDC15* gene and for mapping vegetatively expressed transcription units in the 23 kb of DNA that spanned from *CEN1* to *CDC15* and included *ADE1*. This region was found to be rich in expressed sequences. It gives rise to a total of seven polyadenylated [poly(A)$^+$] transcripts and at least two tRNAs and contains a Ty1 element. This characterization and our earlier observations on the region surrounding *CDC24* (11) provide the basis for an analysis of the gene-coding capacity of this chromosomal region and the entire *S. cerevisiae* genome. Our results are consistent with the idea that chromosome I contains the predicted number of transcribed sequences. Hence, the discrepancy between the physical and genetic estimates of gene number does not appear to be due to a lack of expressed DNA.

MATERIALS AND METHODS

Organisms, bacteriophage, and plasmids. *S. cerevisiae*, *E. coli*, and λ bacteriophages were grown as described previously (11). The *S. cerevisiae* strains used are listed in Table 1. *E. coli* strains SF8 (40), HB101 (34), and 5346 (53) were used to maintain and amplify plasmids. *E. coli* C600 (34) was the host for recombinant λ bacteriophages. An *S. cerevisiae*-λMG14 recombinant DNA library consisting of partially *Sau*3A-digested and size-fractionated 15- to 20-kb DNA from strain AB972 was a gift from M. Y. Graham and M. V. Olson. A similarly constructed partial *Sau*3A library in YCp50 from *S. cerevisiae* GRF88 was a gift from Peter Novick, Jim Thomas, and Mark Rose. Plasmids pBR322, YCp50 (27), YRp7 (5), YRp17 (48), YEp13 (6), and YEp13(*ADE1*)1 (12) have been described previously.

Purification of nucleic acids. Plasmid and bacteriophage λ DNA was prepared by published procedures (34). Yeast DNA for transformation of *E. coli* was prepared by the procedure of Struhl et al. (51). Yeast DNA for genomic Southern blotting experiments was isolated from nuclei prepared by the method of Groner and Phillips (19) from 200 to 1,000 ml of late-exponential-phase YEPD (1.0% [wt/vol] yeast extract, 2.0% [wt/vol] peptone, 2.0% [wt/vol] dextrose [46])-grown cells. Nuclei were suspended in 2 to 10 ml of 1 M NaCl–100 mM Tris–100 mM EDTA, pH 8.0. Sodium dodecyl sulfate was added to 5 mg/ml, and the mixture was heated to 65°C for 10 to 20 min. The extract was then digested sequentially at 37°C for 2 to 8 h each with proteinase K (200 μg/ml), with a heat-treated (90°C, 10 min) mixture of RNase A (50 μg/ml) and RNase T_1 (10 U/ml), and finally with proteinase K (50 μg/ml). DNA was further purified by CsCl density gradient centrifugation as described previously (45). Total and poly(A)$^+$ RNAs were isolated as described previously (30) from exponential-phase *S. cerevisiae* Y185 grown in YEPD medium.

Genetic techniques. Genetic manipulations with *S. cerevisiae* were carried out by standard procedures (46). DNA transformation of *S. cerevisiae* (24) was performed by the method of Ito et al. (26) with 0.3 M LiCl. Unless otherwise noted, phenotypic stability tests for *CEN*-containing plas-

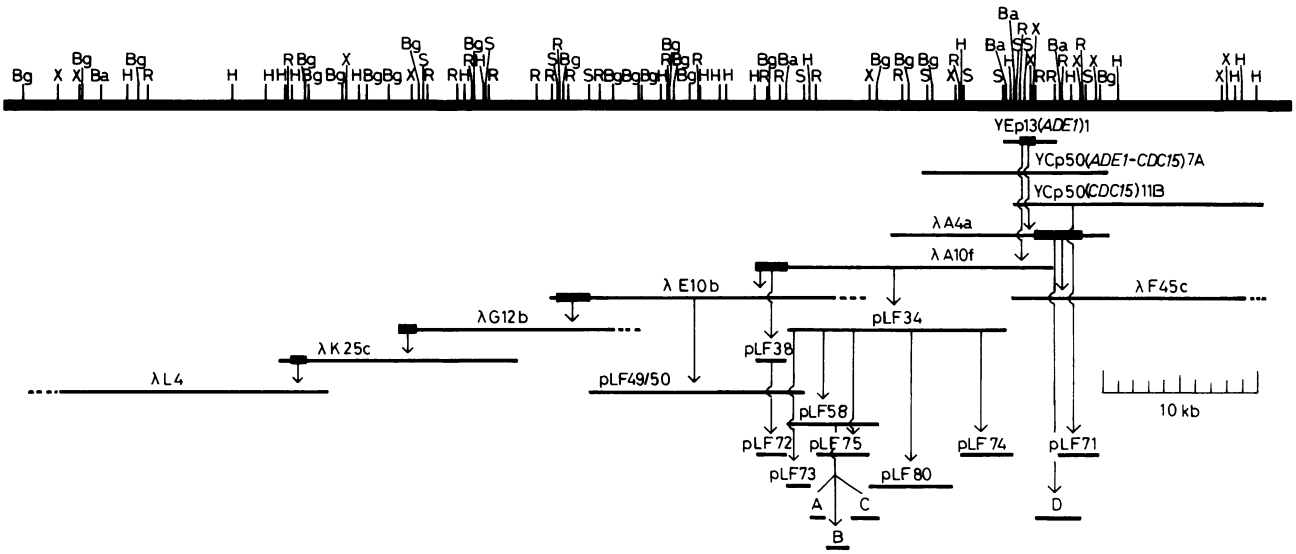

FIG. 1. Molecular cloning of DNA from the region surrounding the *ADE1* gene. DNA was isolated by overlap hybridization as described in the text. Hybridization probes (heavy black boxes) were bounded by the restriction sites noted above. Arrows indicate the λMG14 bacteriophage isolated with each probe. Only approximate ends (broken lines) were determined for inserts in λL4, λG12b, λE10b, and λF45C. *Bam*HI sites created during library construction terminated both ends of the insert in λK25c and the left ends of λG12b and λA10f. Plasmid YEp13(*ADE1*)1 was described previously (12), and YCp50(*ADE1-CDC15*)7A and YCp50(*CDC15*)11B were isolated by complementation of *cdc15-1* mutants as described in the text. Plasmid subclone inserts and purified restriction fragments (solid lines) derived from λ bacteriophages and plasmids are shown below their sources (remaining arrows). Borders correspond to the restriction sites noted above. Plasmids pLF34, pLF38, pLF49, and pLF50 were constructed by using plasmid YRp7 (5) and pLF58, pLF71, pLF72, pLF73, pLF74, pLF75, and pLF80 were constructed by using pBR322 (34). Abbreviations: R, *Eco*RI; S, *Sal*I; Bg, *Bgl*II; H, *Hin*dIII; Ba, *Bam*HI; X, *Xho*I.

mids were carried out by taking cells from agar plates containing selective synthetic defined medium lacking tryptophan or uracil (46), growing them for 12 to 16 cell doublings in YEPD medium, plating 500 to 1,000 cells onto YEPD agar (2.0%, wt/vol) plates, replicating the resulting colonies to selective medium lacking tryptophan or uracil, and determining the percentage of plasmid-retaining Trp^+ or Ura^+ colonies.

Recombinant DNA techniques. DNA was digested with restriction endonucleases as specified by the manufacturers. Agarose gel electrophoresis, restriction mapping, and ligation of DNA fragments were done by standard procedures (34). Restriction fragments were purified by electroelution into a dialysis bag (34), followed by passage of the eluate through a 1.0-ml column of Dowex AG50W-X8 resin equilibrated with 10 mM Tris (pH 7.5)–1.0 mM EDTA. ^{32}P labeling of DNA was carried out as described by Rigby et al. (41). Formation, stabilization, and analysis of R-loop-containing DNA were performed as described previously (28).

DNA and RNA blot hybridization. *S. cerevisiae* DNA (1 to 10 μg per lane) or plasmid or bacteriophage λ DNA that had been digested with appropriate restriction endonucleases (0.25 to 0.5 μg per lane) was subjected to electrophoresis in 0.7% (wt/vol) agarose gels, blotted to nitrocellulose with 3.0 M NaCl–0.3 M sodium citrate (38), and hybridized to ^{32}P-labeled DNA probes as described previously (47). RNA blot hybridization experiments were carried out as described previously (30) with either 2.0 μg of poly(A)$^+$ RNA or 20 μg of total RNA per gel lane.

Isolation of additional DNA from chromosome I. Sequences flanking previously cloned sequences were obtained by overlap hybridization (2, 9). Gel-purified ^{32}P-labeled restriction fragments (Fig. 1) were used as probes for plaque filter hybridization as described by Benton and Davis (3). First, a 1.1-kb *Eco*RI fragment from plasmid YEp13(*ADE1*)1 containing part of the cloned *ADE1* gene from chromosome I (12) was used to probe an *S. cerevisiae*-λMG14 recombinant DNA library. Three bacteriophages were found that had partially overlapping restriction maps that included some of the sequences present in YEp13(*ADE1*)1. These sequences were best defined by the overlapping inserts in λA4a and λA10f. The noted distal restriction fragment from each of these bacteriophages was isolated and used to rescreen the λMG14 library to obtain more distal sequences. The probe from λA10f yielded six partially overlapping bacteriophages, including λE10b, which contained the most new sequences. The probe from the new end of λE10b resulted in nine more bacteriophage isolates that were derived from chromosome I DNA, including λG12b. In addition, this probe hybridized to three bacteriophages that contained inserts that were not derived from chromosome I DNA. These bacteriophages were not investigated further. A unique probe derived from λG12b was isolated. This probe yielded nine bacteriophages, including λK25c. The probe from λK25c gave four isolates, including λL4. These bacteriophages together contained 68 kb of contiguous DNA. On the other side, the probe from λA4a yielded more than 60 recombinant bacteriophages. However, only two contained substantial amounts of new DNA, of which λF45c had the most, approximately 14 kb. In total, 82 kb of contiguous DNA sequences from chromosome I were isolated in this "chromosome walk."

To verify that the newly cloned sequences were derived from chromosome I, gene dosage Southern blot experiments with the ^{32}P-labeled probes used for overlap hybridization were performed as described previously (11, 22). DNA was from strains that were either monosomic for chromosome I and diploid for all other chromosomes (2N-1; X1221a-7c), diploid for all chromosomes (2N; DK8), or disomic for chromosome I and haploid for all other chromosomes (N+1;

X3402-15C). The relative intensities of autoradiographic bands were determined with a Zeineh scanning densitometer (Biomed Instruments, Fullerton, Calif.) with an automatic integrator and autoradiograms that were exposed in the linear range of the film. Fragments that hybridized with the expected ratio of 1:2:4 to the 2N-1:2N:N+1 strains were accepted as being derived from chromosome I (data not shown).

Molecular cloning of the *CDC15* gene. The *CDC15* gene from *S. cerevisiae* was cloned by complementation of the *ts* lethal *cdc15-1* mutation. Strain DK329-4D (*MATα cdc15-1 ade1-1 ura3-1*) was transformed with 60 μg of DNA from the *S. cerevisiae*-YCp50 recombinant DNA library. More than 6,000 Ura^+ colonies were selected on synthetic defined medium lacking uracil at the permissive temperature (25°C). These colonies were then assayed for *cdc15-1* by testing for growth at the restrictive temperature (37°C) on medium lacking uracil. Twenty-seven Ura^+ colonies that grew at 37°C (Ts^+) were selected. To determine whether the Ura^+ Ts^+ phenotype was due to the presence of a plasmid, 20 putative transformants were tested for phenotypic stability after growth on nonselective medium (YEPD). Ten showed simultaneous loss of both the Ura^+ and Ts^+ phenotypes at appropriate levels (10) for *CEN*-containing plasmids. Two of these transformants, designated 7A and 11B, were selected for further use. The other 10 transformants segregated Ura^- colonies that were still Ts^+. These transformants presumably were *cdc15-1* revertants that contained the plasmid vector YCp50 and were not investigated further.

Plasmids contained in yeast transformants 7A and 11B were amplified in *E. coli* HB101 and shown to have partially overlapping restriction maps. The restriction maps suggested that the two plasmid inserts were derived from the region of chromosome I defined by the inserts in bacteriophages λA4a and λF45c (Fig. 1). When transformed back into *S. cerevisiae* DK329-4D, both plasmids again complemented the *cdc15-1* mutation. Furthermore, the plasmid from transformant 7A also complemented the *ade1* mutation present in this yeast strain. Inspection of the restriction map of this plasmid indicated that it overlapped the restriction map of YEp13(*ADE1*)1. In addition, meiotic and mitotic segregation studies performed as described by Clarke and Carbon (10) suggested that both the Ade^+ and Ts^+ phenotypes were genetically linked to the plasmid-borne *URA3* marker (data not shown). These results indicated that the plasmid from yeast transformant 7A also contained the *ADE1* gene. The two plasmids analyzed above were appropriately named YCp50(*ADE1-CDC15*)7A and YCp50(*CDC15*)11B, respectively.

Localization of centromeric DNA from chromosome I (*CEN1*). DNA from the appropriate region of chromosome I was subcloned in the autonomously replicating vector YRp7 to give plasmids pLF34, pLF49, and pLF50 (Fig. 1). To find which plasmid contained *CEN1*, the above plasmids and the vector YRp7 were transformed into *S. cerevisiae* DK357-3A and tested for mitotic stability under nonselective conditions as described above. Plasmids pLF49 and pLF50 exhibited the stability appropriate for a *CEN*-containing plasmid (see Results). To further localize *CEN1*, plasmid pLF49 was digested with either *Bgl*II or *Eco*RI. The *Bgl*II fragments were ligated into the *Bam*HI site of YRp7, and the *Eco*RI fragments were ligated into the *Eco*RI site of the autonomously replicating vector YRp17. The two pools of subclones were amplified in *E. coli* and transformed into *S. cerevisiae* DK357-3A. Trp^+ transformant colonies were selected on plates lacking tryptophan and examined for mitotic stability by a modification of the procedure described by Hsiao and Carbon (25). Transformant colonies selected on plates lacking tryptophan were subjected to three successive cycles of replica plating from nonselective YEPD medium to selective medium lacking tryptophan. Colonies growing on selective medium after this selection were analyzed. Most of the original Trp^+ transformants did not survive the selection, probably because they contained a plasmid that lacked a centromeric sequence. Colonies that remained were tested for their ability to retain a plasmid after 12 to 16 generations of nonselective growth on YEPD medium. Plasmids from stable transformants were amplified in *E. coli* SF8, isolated, and characterized by standard procedures (34).

RESULTS

Isolation of additional DNA from chromosome I. Overlap hybridization starting from the cloned *ADE1* gene (12) was used to isolate a total of 82 kb of contiguous DNA from chromosome I as described in Materials and Methods. Restriction mapping and gene dosage Southern blots (11, 22) were used to verify the chromosome I origin of the newly cloned sequences (data not shown). The restriction map of the overlapping chromosome I inserts is shown in Fig. 1.

Localization of the *CDC15* gene. The *CDC15* gene maps approximately 2 cM centromere-distal to the *ADE1* gene (37). Based on the appropriate relationship of physical to genetic distances of 3 kb per cM in *S. cerevisiae* (36, 37), this gene should be present on the already cloned segments from chromosome I. To localize *CDC15*, two plasmids that complemented the *cdc15-1* mutation were isolated from an *S. cerevisiae*-YCp50 recombinant DNA library as described in Materials and Methods. The two plasmids, YCp50(*CDC15*)11B and YCp50(*ADE1-CDC15*)7A, had partially overlapping restriction maps that also overlapped the restriction map from the chromosome walk (Fig. 1). Plasmid YCp50(*ADE1-CDC15*)7A also contained the nearby *ADE1* gene (see Materials and Methods).

Electron microscopy of R-loop-containing DNA suggested that plasmids YCp50(*CDC15*)11B and YCp50(*ADE1-CDC15*)7A contained only a single intact transcribed region in the 6 kb of DNA common to both inserts (Fig. 2). The 8-kb *Bgl*II fragment from YCp50(*CDC15*)11B contained an approximately 3.0-kb-long R-loop at one of its ends. Since the other end of this fragment contained plasmid pBR322 sequences, this R-loop could be oriented unambiguously. A second R-loop located at the insert-vector junction was also seen on the 8-kb *Bgl*II fragment. This R-loop was due to part of the previously localized (12) *ADE1* gene. Additional R-loop electron microscopy with *Bam*HI-cleaved plasmid YCp50(*CDC15*)11B confirmed the previous result showing that the end of the putative *CDC15* transcribed region mapped very close to the *Bgl*II site. All the other transcribed sequences on YCp50(*CDC15*)11B were outside the region shared with YCp50(*ADE1-CDC15*)7A. These transcribed sequences, which map to the right of the *CDC15* gene, were not accurately mapped or investigated further. RNA blot hybridization experiments later established that the size of the putative CDC15 RNA was actually 3.5 kb (see below).

To show that this transcribed region contained the bona fide *CDC15* gene, it was disrupted in the genome by one-step gene replacement (42) with the *S. cerevisiae LEU2* gene as described in the legend to Fig. 2. Gene replacement carried out in strain DK358, a *CDC15/cdc15-1* heterozygote, yielded Leu^+ transformants that either did not grow at 37°C on YEPD medium (Ts^-) or grew under these conditions (wild

type [WT]. The Ts$^-$ phenotype was due to uncovering the mutant *cdc15-1* gene by deleting the WT copy of this gene. Genetic analysis of a Ts$^-$ transformant (DK358Ω1) indicated the presence of a recessive lethal mutation that was associated with *LEU2*, allelic to *cdc15-1*, and linked to *ADE1*. Five asci contained two viable *ade1 cdc15-1 leu2* spores and two inviable spores, and five asci contained one viable *ade1 cdc15-1 leu2* spore and three inviable spores. The third inviable spore in these asci was probably due to normal cosegregation of several unlinked genes that were not related to the *CDC15* gene. This inviability was not considered significant. The inviable spores from the asci with two viable spores were examined by light microscopy 24 to 48 h following dissection. These spores appeared to germinate and bud but did not give rise to two separate cells. This phenotype resembles the terminal phenotype of the Ts$^-$ *cdc15-1* mutants (20).

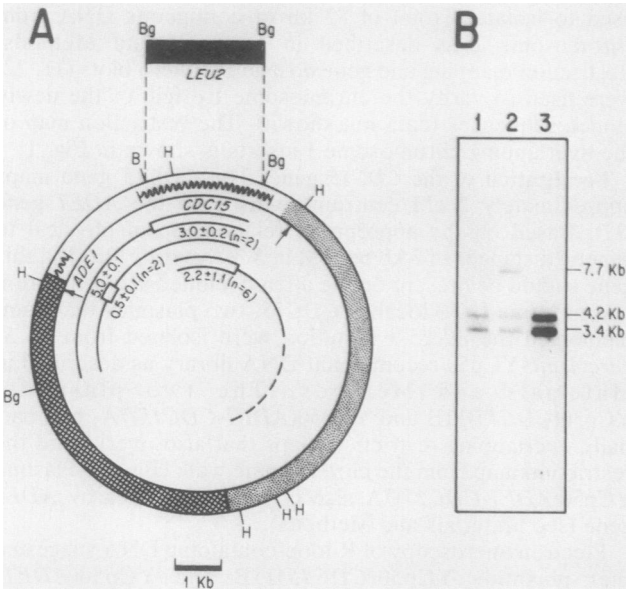

FIG. 2. Location of the *CDC15* gene. (A) Transcript mapping and construction of a deletion in *CDC15*. The locations of the transcribed regions (wavy line) on YCp50(*CDC15*)11B were determined by electron microscopy of R-loop-containing DNA as described in the text. This plasmid contained the *CDC15* gene and part of the *ADE1* gene inserted in the vector YCp50 (cross-hatched box). The sizes (in kilobases ± the standard deviation) of the regions containing an R-loop (open box) and the region of the insert that was not present in YCp50 (*ADE1-CDC15*)7A (stippled area) are shown. To construct a deletion, the 2.6-kb *Bam*HI-*Bgl*II fragment from the *CDC15* transcribed region was deleted from the plasmid by complete digestion with *Bam*HI followed by partial digestion with *Bgl*II. The resultant 20-kb fragment was gel purified and ligated to the 2.75-kb *Bgl*II fragment containing the *S. cerevisiae LEU2* gene purified from plasmid YEp13. The ligation mixture was transformed into *E. coli* 5346 (LeuB$^-$) and Leu$^+$ ampicillin-resistant colonies were selected. Plasmid DNA was isolated from the *E. coli* transformants, digested with *Hin*dIII, and transformed into *S. cerevisiae* DK358. Leu$^+$ transformants were selected and analyzed for a disrupted copy of *CDC15* as described in the text. Restriction site symbols are the same as described in the legend to Fig. 1, except B, *Bam*HI. (B) Blot hybridization analysis of the disrupted *CDC15* gene. DNA isolated from the strains noted below was digested with *Hin*dIII, subjected to electrophoresis in agarose gels, blotted to nitrocellulose, and probed with a mixture of the 4.2-kb and 3.4-kb *Hin*dIII fragments from YCp50(*CDC15*)11B (see arrows in panel A). Lane 1, Control DK358; lane 2, DK358Ω1 (*cdc15::LEU2/cdc15-1*); lane 3, DK358Ω1-5A, viable Leu$^-$ haploid from DK358Ω1.

Genetic analysis of two of the WT transformants suggested that both were due to gene conversion at *leu2* (24). These transformants produced asci in which all four spores were viable and the *LEU2* gene showed no linkage to *ADE1*. These transformants were not analyzed further. Transformants due to gene replacement of the *cdc15-1* allele that would have left the WT copy intact were not found in this limited search.

Genomic DNA blot hybridization experiments showed that the Ts$^-$ transformant DK358Ω1 contained the 7.7-kb *Hin*dIII fragment indicative of gene replacement. This fragment was not found in the untransformed diploid parent or in viable haploid segregants derived from the transformant. In addition, all strains examined contained a 3.4-kb and a 4.2-kb *Hin*dIII fragment due to a resident undisrupted copy of *cdc15-1* (Fig. 2B). [Coincidentally, plasmid YCp50(*CDC15*) 11B also contained a 4.2-kb *Hin*dIII fragment due to a site within the vector DNA.]

In total, these results indicate that the disrupted 3.5-kb transcribed region corresponds to the bona fide *CDC15* gene. As the *CDC15* gene is centromere-distal to *ADE1*, the molecular localization of the *CDC15* gene permits the orientation of the cloned sequences with respect to the genetic map (see Fig. 4).

Physical localization of centromeric DNA from chromosome I (*CEN1*). Since the *ADE1* gene is closely linked to the chromosome I centromere (36, 37), *CEN1* sequences were expected to be on the cloned DNA. Since *ADE1* is on the right arm, *CEN1* DNA should be to the left of this gene. To identify the *CEN1* sequences, cloned fragments mapping to the left of *ADE1* were tested for their ability to stabilize the inheritance of autonomously replicating vectors in *S. cerevisiae* (10) as described in Materials and Methods. The 14-kb *Sal*I fragment from λE10b imparted mitotic stability to YRp7 in either orientation (pLF49 and pLF50, Fig. 1). Ninety-three percent (±3%, n =5) of the cells retained plasmid pLF49, and 92 ± 2% (n = 4) of the cells retained plasmid pLF50 and gave Trp$^+$ colonies following 12 to 16 cell divisions on nonselective medium (YEPD). In contrast, transformants containing YRp7 either with the 13.7-kb *Bam*HI fragment from the region 0.5 kb to the left of the *ADE1* gene (pLF34, Fig. 1) or without any insert at all showed almost total loss of plasmid. Less than 1.0% of the colonies were Trp$^+$ after 12 to 16 cell divisions on YEPD medium. These results suggested that *CEN1* was contained in the 14-kb *Sal*I fragment.

To further localize *CEN1*, YRp7 or YRp17 plasmid subclones derived from pLF49 which contained the 14-kb *Sal*I fragment were transformed in *S. cerevisiae*. Three plasmids that exhibited mitotic stability were isolated as described in Materials and Methods. Restriction mapping of the three plasmids indicated that each contained DNA from the same region. Two contained the *Bgl*II fragment, while one contained the *Eco*RI fragment shown in Fig. 3. These experiments placed *CEN1* inside the 4.3-kb *Eco*RI fragment (Fig. 3). Genetic analysis performed as described by Clarke and Carbon (10) showed that this DNA enabled plasmid YRp17 to segregate during meiosis (V. Guacci and D. Kaback, unpublished observations).

CEN1 has been shown independently to reside on a 1.7-kb *Hin*dIII fragment (23). These results are consistent with ours, since the region we defined also contained this *Hin*dIII fragment. The orientation of this *Hin*dIII fragment with respect to the rest of the physical map was determined by

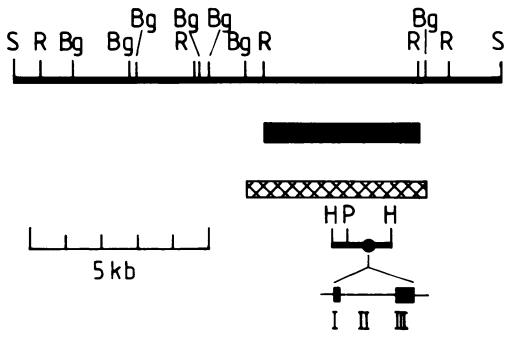

FIG. 3. Localization of *CEN1* DNA. *CEN1*-containing restriction fragments that stabilize the inheritance of autonomously replicating plasmid vectors in *S. cerevisiae* were isolated as described in the text. The top line shows the 14-kb *Sal*I fragment from λ E10b contained in plasmids pLF49 and pLF50; black box, the 4.3-kb *Eco*RI fragment inserted in YRp17; hatched box, the 4.7-kb *Bgl*II fragment inserted in YRp7. The precise location of *CEN1* (●) with respect to these larger chromosome fragments was determined by orienting the *Pvu*II site (P) in the 1.7-kb *Hin*dIII fragment that was shown independently to contain *CEN1* (23). The location of the centromeric consensus sequences (I, II, III [23]) is shown. Other restriction site symbols are the same as in Fig. 1.

mapping the *Pvu*II site with respect to sites flanking the *Hin*dIII fragment (Fig. 3). As the orientation of the centromere consensus sequences on the *Hin*dIII fragment has also been determined (23), we were able to place *CEN1* at its precise position on the physical map (Fig. 3 and 4).

Transcripts in the *CEN1-ADE1-CDC15* region. The number and location of transcribed sequences in the *CEN1-ADE1-CDC15* region (Fig. 4) was determined by a combination of RNA blot hybridization experiments and electron microscopy of R-loop-containing DNA. To facilitate these studies, most of the region was subcloned into pBR322 as described in the legend to Fig. 1. Northern blots containing either total or poly(A)$^+$ RNA indicated that this DNA contained a total of seven different transcribed regions that gave rise to poly(A)$^+$ RNA and a Ty1 element. In addition, two regions were complementary to low-molecular-weight (4S) RNA species present in the total RNA lanes but not the poly(A)$^+$ RNA lanes. These sequences probably correspond to tRNA genes.

A 1.9- and a 0.7-kb poly(A)$^+$ transcript hybridized to the 2.0-kb *Bam*HI fragment present in pLF72, while only a 1.9-kb transcript hybridized to the neighboring 1.5-kb *Bam*HI-*Hin*dIII fragment present in pLF73. This suggested that the 1.9-kb transcript came from the region to the right of the 0.7-kb transcript (Fig. 4).

Five transcripts hybridized to the 3.4-kb *Eco*RI-*Xho*I fragment present in plasmid pLF75. First, there were a 2.1-kb and a 1.0-kb poly(A)$^+$ RNA species. The relative positions of these transcripts were determined with the gel-purified probes marked A, B, and C from pLF58 (Fig. 4). These probes overlap the region defined by the pLF75 insert. Probe A hybridized to the 2.1-kb transcript, while probe B hybridized to both the 2.1- and 1.0-kb transcripts. These results allowed the positioning of the 2.1-kb transcript to the left of the 1.0-kb transcript. In addition, probe mixing experiments clearly showed that the 2.1-kb transcript clearly differed in size from the 1.9-kb transcript found with pLF72. Therefore, these RNA species were not derived from the same transcribed region. The other three transcripts were 5.7, 0.8, and 0.6 kb in length and hybridized only to probe C, suggesting that they mapped to the right of the 1.0-kb transcript. The presence of a 5.5-kb *Xho*I fragment containing the additional restriction sites shown (Fig. 4) suggested the presence of a Ty1 repetitive element. This Ty1 element was confirmed by showing that it hybridized to cloned Ty1 DNA (data not shown). The pLF80 insert should be completely internal to the Ty1 element (16). pLF80 also hybridized to 5.7-, 0.8-, and 0.6-kb RNA species (Fig. 4). Transcripts of this size have been shown previously to be complementary to Ty1 elements (16). These results suggested that these transcripts were indeed from a Ty1 ele-

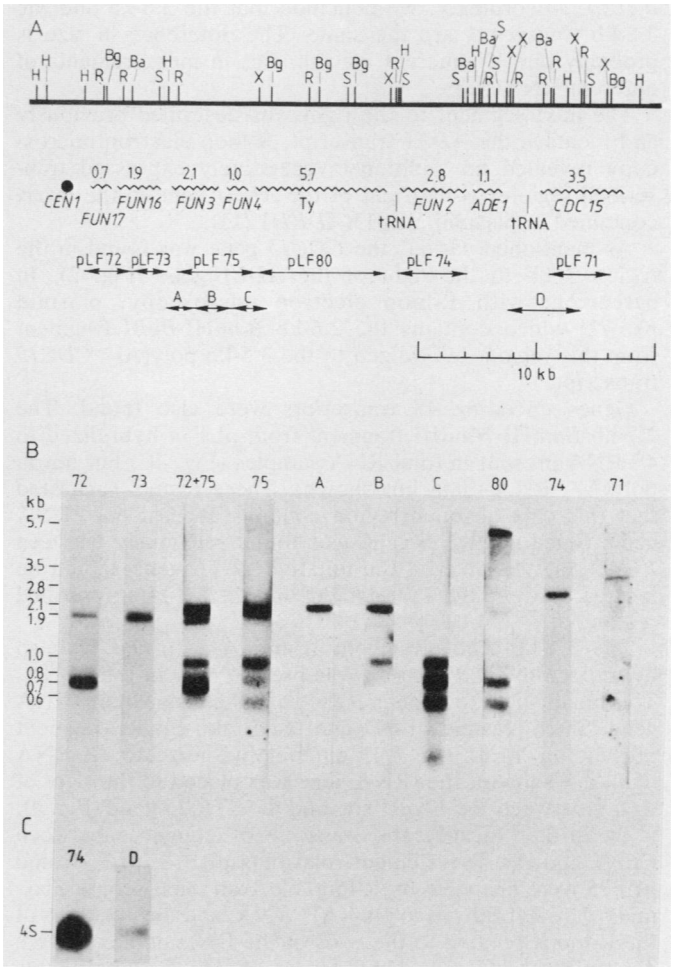

FIG. 4. Transcribed sequences in the *CEN1-ADE1-CDC15* region. (A) Restriction map with the approximate sizes, locations, and identities of transcribed regions. The size and location of *ADE1* and *FUN2* are from a previous report (12). The borders of the hybridization probes are indicated by the double-headed arrows. Placement of transcribed regions on the map also is based on R-loop electron microscopy (Fig. 2 and 5). Transcribed regions (wavy lines) were positioned symmetrically around the center of the R-loop by using transcript sizes determined by blot hybridization. (B) Blot hybridization with poly(A)$^+$ RNA. RNA was prepared, fixed to diazobenzoxymethyl paper and hybridized to ^{32}P-labeled probes (noted above each lane) as described previously (30). Experiments used parallel lanes from a single gel. (C) Blot hybridization with total RNA. Experiments were performed as described above except the two blots were prepared and probed separately with DNA labeled to different specific activities. Only the lower part of the blots is shown.

ment, although they were not necessarily transcribed from this particular Ty1 element.

The 5.7-kb transcript is not visible in the autoradiograph shown in Fig. 4C. However, the absence of this band was not significant, since longer exposures of this blot clearly indicated the presence of the 5.7-kb transcript with probe C. Most likely the absence of this band in this particular blot was due to poor transfer of high-molecular-weight RNA or an experimental artifact.

Proceeding to the right, the 2.7-kb *Bam*HI-*Hin*dIII fragment contained in pLF74 hybridized to a 2.8-kb RNA species. This region overlaps a region in YEp13(*ADE1*)1 that was found previously to be complementary to both the *ADE1* transcript and a 2.6-kb transcript (formerly designated *a* [12]). Accordingly, we conclude that the 2.6-kb and the 2.8-kb transcripts are the same. The difference in size is probably due to inherent inaccuracies in measurements of different RNA blots.

The next segment to the right was described previously and includes the *ADE1* transcript. R-loop electron microscopy revealed no additional vegetatively expressed transcribed regions to the right of the *ADE1* gene in the insert contained in plasmid YEp13(*ADE1*)1 (12).

As mentioned earlier, the *CDC15* gene was found in the region 2 kb to the right of the *ADE1* gene (Fig. 2). In agreement with R-loop electron microscopy, plasmid pLF71, which contains the 2.6-kb *Bam*HI-*Bgl*II fragment from this region, hybridized to the 3.5-kb poly(A)$^+$ *CDC15* transcript.

Genes encoding 4S transcripts were also found. The 2.7-kb *Bam*HI-*Hin*dIII fragment from pLF74 hybridized to 4S RNA present in total RNA samples (Fig. 4C) but not in poly(A)$^+$ RNA (data not shown). These results suggested that this chromosomal region contains at least one tRNA gene. Since no tRNA gene was found previously between *FUN2* and *ADE1* (12), the putative tRNA gene should be located between the Ty1 element and the 2.8-kb transcribed region.

The 3.1-kb *Xho*I fragment from λA4a (probe D) also hybridized to 4S RNA that was present only in total RNA. This result suggested the presence of at least one more tRNA gene. Since plasmid pLF71 and the 1.1-kb *Eco*RI fragment present on YEp13(*ADE1*)1 did not hybridize to 4S RNA (data not shown), the tRNA gene was placed to the right of *ADE1* between the *Eco*RI site and the *CDC15* gene (Fig. 4).

To further localize the transcribed sequences between *CEN1* and the Ty1 element, plasmids pLF72, pLF73, and pLF75 were analyzed by R-loop electron microscopy. Plasmids were hybridized to poly(A)$^+$ RNA, and the positions of the R-loops relative to the ends of the DNA duplexes were determined as described in the legend to Fig. 5. Four regions were found that gave rise to poly(A)$^+$ transcripts. The results were in good agreement with the results obtained in the RNA blot hybridization experiments and permitted more accurate mapping of transcribed regions with respect to the noted restriction sites.

In summary, besides the transcription units that corresponded to the *ADE1* and *CDC15* genes and the Ty1 element, a total of five transcribed regions that gave rise to poly(A)$^+$ RNA and did not correspond to known genes or chromosomal elements were found. These regions have been temporarily designated *FUN* genes because their function is unknown now (11). These genes have been consecutively numbered by their order of discovery, starting with *FUN2*, which is the transcribed region previously designated *a* (2.8-kb transcript) (12).

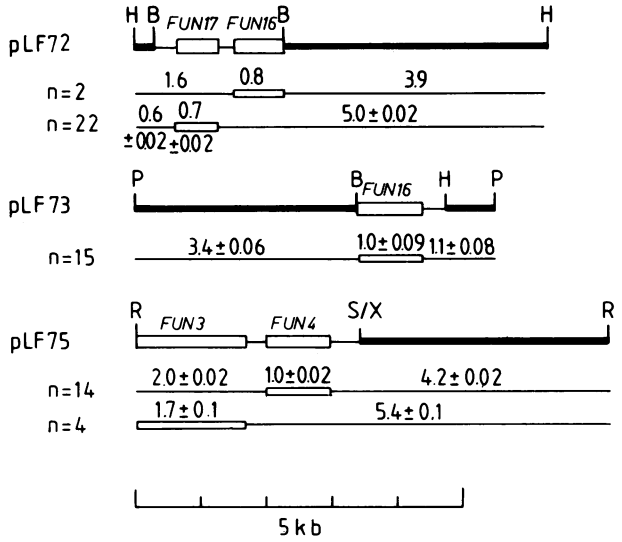

FIG. 5. Mapping of transcribed regions between *CEN1* and the Ty1 element by electron microscopy of R-loop-containing DNA. Plasmid pLF72, pLF73 or pLF75 DNA (250 ng) was cleaved with the noted restriction enzyme, crosslinked once per 2 to 5 kb with Trioxsalen and UV light, and hybridized to 5.0 μg of poly(A)$^+$ RNA in a 50-μl reaction mixture. The reaction mixture was treated with 1.0 M glyoxal and prepared for electron microscopy, and R-loop-containing DNA was analyzed. Heavy black line, Vector sequences; thin black line, *S. cerevisiae* insert DNA; open boxes, transcribed regions. A compilation of the length measurements (± standard deviation where *n* is the number of molecules analyzed) made on each type of molecule with an R-loop is shown. Plasmids were cleaved so that all *S. cerevisiae* DNA was contained on one side of the linearized molecule and occupied less than half of its total length. Accordingly, all R-loops were assumed to be present on *S. cerevisiae* sequences and could be positioned unambiguously with respect to the noted restriction site. *FUN* designations are noted. *CEN1* is approximately 1 kb to the left of *FUN17*. *FUN16* was bissected by the *Bam*HI site and was located on plasmids pLF72 and pLF73. Abbreviations: B, *Bam*HI; H, *Hin*dIII; P, *Pst*I; R, *Eco*RI; S/X, *Sal*I-*Xho*I junction.

DISCUSSION

To continue our systematic study of the molecular organization of chromosome I, 82 kb of DNA surrounding the *ADE1* gene was isolated and analyzed. The positions of *CEN1* DNA and the *CDC15* gene were determined. In addition, transcribed regions that are expressed in vegetative cells in the 23 kb of DNA that spanned from *CEN1* through the *CDC15* gene were characterized. A total of seven regions that gave rise to poly(A)$^+$ transcripts and at least two regions that contained one or more tRNA genes were demonstrated. This region also harbored a Ty1 element.

At least 18.7 out of the 23 kb investigated, or 80% of this DNA, appeared to be transcribed. This value is higher than the average of 60% found so far for *S. cerevisiae* (11). Even this density of poly(A)$^+$ transcription units should be considered a minimal estimate, since only genes expressed under a very limited set of conditions were examined (diploid cells from a single strain, Y185, growing exponentially in rich medium [YEPD] at 30°C). Other transcripts from this region might be expressed under different circumstances, e.g., in cells grown under different conditions or in strains with different genetic backgrounds. Indeed, preliminary evidence indicates the existence of a sporulation-induced 1.4-kb transcribed region located between *ADE1* and

CDC15. This segment did not hybridize to any vegetative poly(A)$^+$ RNA species. In addition, the 0.5-kb region located just to the right of *CEN1* has not yet been examined for transcripts. Accordingly, it is possible that even this region might harbor one or more additional transcription units. At least one other region, that surrounding the *HIS3* gene, appears to be equally packed with transcribed regions (50). Thus, there appear to be parts of the yeast genome which resemble bacterial genomes in the density of transcripts.

A consequence of this high gene density is that only limited space is available for regulatory elements such as upstream activation sites. Although normally short, these sequences are known to be located up to 2 kb in front of the sequences on which they act (17; A. Kempers-Veenstra, personal communication). The organization of sequences in most of the region described precludes such long-range interactions unless the regulatory elements overlap other transcribed regions.

These data together with those from the previously characterized *CDC24* region from chromosome I, amount to a total of 15 transcribed regions on 41 kb of DNA (not counting tRNA genes and counting Ty1 as a single transcribed region) that have been characterized so far. This DNA encompasses approximately 16% of chromosome I. If we assume that this gene density is typical for the entire length of this chromosome, chromosome I would contain 98 genes. This value is similar to that predicted previously (11). It is possible that the two regions examined to date were predisposed to genetic activity and that the rest of the chromosome is more devoid of genes. This possibility is considered unlikely. Large regions of nontranscribed DNA were never found when examining R-loops made with total genomic DNA and poly(A)$^+$ RNA from *S. cerevisiae* (28). Studies in progress on cloned chromosome I DNA will reveal whether these two regions are typical.

Examination of additional parts of chromosome I (unpublished data) already has suggested that the first two regions examined are indeed typical. We found 10 more transcribed regions on an additional 20 kb of DNA (unpublished observations). Since we have already found a total of twice as many transcribed regions as known genes on chromosome I, it is becoming highly unlikely that the small number of genes on chromosome I found by mutational analyses is due to a lack of transcribed DNA. Gene disruption experiments are under way to determine the function of many of the unknown transcribed regions (*FUN* genes). These studies should help approximate the number of essential genes that are present in the genome of *S. cerevisiae*.

In mapping tRNA genes, two loci were found in the 23-kb region examined. Since the hybridization probes were large, it is possible that each tRNA locus contained a cluster of tRNA genes. However, tRNA gene clusters like those found in *Drosophila melanogaster* (55) have not been found in *S. cerevisiae*. While the presence of at least two tRNA genes in 23 kb of DNA is higher than the average frequency of one tRNA gene per 40 kb (45), a random distribution of tRNA genes in the genome should give several 23-kb regions with two or more tRNA genes. Accordingly, there is no evidence that the distribution of tRNA genes in this region is unusual.

One of the two tRNA loci was located within 300 bp of a Ty1 element. As tRNA genes often are located 0 to 500 base pairs from Ty1 or solo δ or σ sequences (13, 15, 18, 43), the tRNA locus between the Ty1 element and the *FUN2* gene is another example of this correlation. In contrast, the tRNA gene between *ADE1* and *CDC15* does not appear to be close to any repetitive sequence.

These studies required the cloning of the *S. cerevisiae* *CDC15* gene. This gene is required for the completion of nuclear division and has been implicated in the process of mitotic chromosome segregation (20, 21). However, the precise function of the *CDC15* gene product is not known. It is worth noting that our initial attempts to isolate this gene by complementation with libraries contained in high-copy-number vectors (YRp7 and YEp13) failed. In contrast, *CDC15* was readily cloned from the low-copy-number *CEN4* vector YCp50 library. Similar experiences were encountered when another yeast cell cycle gene, *CDC24*, was cloned (K. Coleman and J. Pringle, personal communication). These results suggest that the *CDC* genes or sequences closely linked to them are toxic to yeast when present in many copies. As the region to the left of the *CDC15* gene showed normal stability in high-copy-number plasmids (12) and the *CDC15* gene was the only intact gene present on both plasmids isolated, it is most likely that any toxicity is due to the *CDC15* gene product itself. The possible deleterious nature of overproduced *CDC15* gene product may have functional significance.

A Ty element was found between *CEN1* and *ADE1*. This element was classified as Ty1 based on its characteristic distribution of *Eco*RI and *Xho*I sites and its cross-hybridization with Ty1 DNA. Several differences in the pattern of Ty1 transcripts were found when this Ty1 DNA was used to probe poly(A)$^+$ RNA from strain Y185. In addition to the previously observed 5.7-kb and 0.8-kb RNA species (16, 54), a 0.6-kb poly(A)$^+$ RNA was observed. It is possible that the 0.6-kb RNA was initiated outside and terminated inside the Ty1 DNA or vice versa. Alternatively, this transcribed region could be completely contained in a Ty1 element. This transcript was not investigated further. Since it is not known whether the Ty1 DNA found here is transcriptionally active, the source of the Ty1 RNA could be another Ty1 element(s).

The data allow a correlation between genetic and physical distance in this region of chromosome I. However, the presence of the Ty1 element complicates the accuracy of any correlation. The average genetic distance between *CEN1* and *ADE1* is 4.2 cM (36). The physical distance from *CEN1* to the center of the *ADE1* transcribed region is 18 kb including the Ty1 element and 12 kb without it. Accordingly, the recombination rate varies from 0.23 cM/kb to 0.35 cM/kb, depending on the presence of the Ty1 element. Without Ty1, it appears that the recombination frequency in the *CEN1-ADE1* region is equal to the average of 0.33 cM/kb for the total yeast genome (36, 37, 49). With Ty1, the recombination rate is approximately 1/3 lower than average. While we do not know whether mapping studies in this region used strains with this Ty1 element, any deviation from the average rate of recombination is much smaller than at other regions on the yeast genome, such as *CDC24-PYK1* (11) and *CEN3-PGK1* (10).

The *ADE1* and *CDC15* genes are separated by 2.2 cM on the genetic map (36), while the centers of their transcribed regions are separated by 3.9 kb. The physical distance separating the mutant alleles used in mapping is not known and could vary from 1.6 to 6.2 kb. Thus, calculating the rate of recombination per kilobase at this time is subject to this variation. If we calculate the rate by measuring the distance between the centers of the two transcribed regions, the value obtained is 0.56 cM/kb. This average is approximately two-fold higher than the average for the yeast genome. Unfortunately, there is no way of knowing whether this increase is significant since it could be due to the position of the mutant

alleles. In total, the recombination rate per kilobase of the whole region appears to show some variation. Similar variations have been observed elsewhere in the yeast genome (49). There is at present no evidence for either a hot spot or a cold spot for recombination in the region of the yeast genome described here.

So far, a total of 123 kb of chromosome I DNA has been cloned. This DNA includes the 29 kb from the *CDC24-PYK1* region (11), the 12 kb from the *MAK16* region (R. Wickner, T. J. Kuo, J. Crowley, J. O'Neil, and D. Kaback, submitted for publication), and the 82 kb reported here. Taken together in the order listed above, these segments form an almost contiguous DNA sequence. Genomic blot hybridization data indicate that an approximately 3-kb segment mapping between *PYK1* and *MAK16* has not yet been cloned (H. Y. Steensma, unpublished results). The segments that have been cloned encompass roughly one-half of the estimated DNA from chromosome I (8, 44) and, based on the genetic map (37), must contain all the genes so far defined by classic means with the exception of *FL01*.

ACKNOWLEDGMENTS

We thank Maynard Olson for the λMG14 library, Peter Novick for the YCp50 library, Carol Newlon for useful discussions, and Brenda Lemon for help in preparing the manuscript.

This work was supported by grants from the National Science Foundation and the New Jersey Cancer Research Commission to D.B.K. and by a travel grant from the Netherlands Organization for the Advancement of Pure Research (Z.W.O.) to Y.S.

LITERATURE CITED

1. **Bachmann, B. J.** 1983. Linkage map of *Escherichia coli* K-12, edition 7. Microbiol. Rev. **47**:180–230.
2. **Bender, W., P. Spierer, and D. S. Hogness.** 1983. Chromosomal walking and jumping to isolate DNA from the *Ace* and *rosy* loci and the bithorax complex in *Drosophila melanogaster*. J. Mol. Biol. **168**:17–33.
3. **Benton, W. D., and R. W. Davis.** 1977. Screening λgt recombinant clones by hybridization to single plaques *in situ*. Science **196**:180–182.
4. **Bossy, B., L. M. C. Hall, and P. Spierer.** 1984. Genetic activity along 315 kb of the *Drosophila* chromosome. EMBO J. **3**:2537–2541.
5. **Botstein, D., S. C. Falco, S. E. Stewart, M. Brennan, S. Scherer, D. T. Stinchcomb, K. Struhl, and R. W. Davis.** 1979. Sterile host yeasts (SHY): a eukaryotic system of biological containment for recombinant DNA experiments. Gene **8**:17–24.
6. **Broach, J. R., J. N. Strathern, and J. B. Hicks.** 1979. Transformation in yeast: development of a hybrid cloning vector and isolation of the *CAN1* gene. Gene **8**:121–133.
7. **Bruenn, J., and R. K. Mortimer.** 1970. Isolation of monosomics in yeast. J. Bacteriol. **102**:548–551.
8. **Carle, G. F., and M. V. Olson.** 1984. Separation of chromosomal DNA molecules from yeast by orthogonal-field-alternation gel electrophoresis. Nucleic Acids Res. **12**:5647–5664.
9. **Chinault, A. C., and J. Carbon.** 1979. Overlap hybridization screening: isolation and characterization of overlapping DNA fragments surrounding the *leu2* gene on yeast chromosome III. Gene **5**:111–126.
10. **Clarke, L., and J. Carbon.** 1980. Isolation of a yeast centromere and construction of functional small circular chromosomes. Nature (London) **287**:504–509.
11. **Coleman, K. G., H. Y. Steensma, D. B. Kaback, and J. R. Pringle.** 1986. Molecular cloning of chromosome I DNA from *Saccharomyces cerevisiae*: isolation and characterization of the *CDC24* gene and adjacent regions of the chromosome. Mol. Cell. Biol. **6**:4516–4525.
12. **Crowley, J. C., and D. B. Kaback.** 1984. Molecular cloning of chromosome I DNA from *Saccharomyces cerevisiae*: isolation of the *ADE1* gene. J. Bacteriol. **159**:413–417.
13. **Cummins, C. M., M. R. Culbertson, and G. Knapp.** 1985. Frameshift suppressor mutations outside the anticodon in yeast proline tRNAs containing an intervening sequence. Mol. Cell. Biol. **5**:1760–1771.
14. **Edgar, R. S., and I. Lieslausis.** 1964. Temperature-sensitive mutants of phage T4D: their isolation and characterization. Genetics **49**:649–666.
15. **Eigel, A., and H. Feldmann.** 1982. Ty1 and delta elements occur adjacent to several tRNA genes in yeast. EMBO J. **1**:1245–1250.
16. **Elder, R. T., E. Y. Loh, and R. W. Davis.** 1983. RNA from the yeast transposable element Ty1 has both ends in the direct repeats, a structure similar to retrovirus RNA. Proc. Natl. Acad. Sci. USA **80**:2432–2436.
17. **Elion, E. A., and J. R. Warner.** 1984. The major promoter element of rRNA transcription in yeast lies 2 kb upstream. Cell **39**:663–673.
18. **Gafner, J., E. M. de Robertis, and P. Philippsen.** 1983. Delta sequences in the 5′ non-coding region of yeast tRNA genes. EMBO J. **2**:583–591.
19. **Groner, B., and S. L. Phillips.** 1975. Polyadenylate metabolism in the nuclei and cytoplasm of *Saccharomyces cerevisiae*. J. Biol. Chem. **250**:5640–5646.
20. **Hartwell, L. H.** 1975. *Saccharomyces cerevisiae* cell cycle. Bacteriol. Rev. **38**:164–198.
21. **Hartwell, L. H., and D. Smith.** 1985. Altered fidelity of mitotic chromosome transmission in cell cycle mutants of *S. cerevisiae*. Genetics **110**:381–395.
22. **Hicks, J., and G. R. Fink.** 1977. Identification of chromosomal location of yeast DNA from hybrid plasmid pY*eleu10*. Nature (London) **269**:265–267.
23. **Hieter, P., D. Pridmore, J. H. Hegemann, M. Thomas, R. W. Davis, and P. Philippsen.** 1985. Functional selection and analysis of yeast centromeric DNA. Cell **42**:913–921.
24. **Hinnen, A., J. B. Hicks, and G. R. Fink.** 1978. Transformation of yeast. Proc. Natl. Acad. Sci. USA **75**:1929–1933.
25. **Hsiao, C.-L., and J. Carbon.** 1981. Direct selection procedure for the isolation of functional centromeric DNA. Proc. Natl. Acad. Sci. USA **78**:3760–3764.
26. **Ito, H., Y. Fukuda, K. Murata, and A. Kimura.** 1983. Transformation of intact yeast cells treated with alkali cations. J. Bacteriol. **153**:163–168.
27. **Johnston, M., and R. W. Davis.** 1984. Sequences that regulate the divergent *GAL1-GAL10* promoter in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **4**:1440–1448.
28. **Kaback, D. B., L. M. Angerer, and N. Davidson.** 1979. Improved methods for the formation and stabilization of R-loops. Nucleic Acids Res. **6**:2499–2517.
29. **Kaback, D. B., M. M. Bhargava, and H. O. Halvorson.** 1973. Location and arrangement of genes coding for ribosomal RNA in *Saccharomyces cerevisiae*. J. Mol. Biol. **79**:735–739.
30. **Kaback, D. B., and L. R. Feldberg.** 1985. *Saccharomyces cerevisiae* exhibits a sporulation-specific temporal pattern of transcript accumulation. Mol. Cell. Biol. **5**:751–761.
31. **Kaback, D. B., P. W. Oeller, H. Y. Steensma, J. Hirschman, D. Ruezinsky, K. G. Coleman, and J. R. Pringle.** 1984. Temperature-sensitive lethal mutations on yeast chromosome I appear to define only a small number of genes. Genetics **108**:67–90.
32. **Kuenzi, M. T., M. A. Tingle, and H. O. Halvorson.** 1974. Sporulation of *Saccharomyces cerevisiae* in the absence of a functional mitochondrial genome. J. Bacteriol. **117**:80–88.
33. **Lauer, G. D., T. M. Roberts, and L. C. Klotz.** 1977. Determination of the nuclear DNA content of *Saccharomyces cerevisiae* and implications for the organization of DNA in yeast chromosomes. J. Mol. Biol. **114**:507–526.
34. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
35. **Mortimer, R. K., and D. C. Hawthorne.** 1973. Genetic mapping in *Saccharomyces*. IV. Mapping of temperature-sensitive genes and use of disomic strains in localizing genes. Genetics **74**:33–54.
36. **Mortimer, R. K., and D. Schild.** 1980. Genetic map of *Saccha-*

romyces cerevisiae. Microbiol. Rev. **44**:519–571.
37. **Mortimer, R. K., and D. Schild.** 1985. Genetic map of *Saccharomyces cerevisiae*, edition 9. Microbiol. Rev. **49**:181–212.
38. **Nagamine, Y., A. Sentenac, and P. Fromageot.** 1980. Selective blotting of restriction DNA fragments on nitrocellulose membranes at low salt concentrations. Nucleic Acids Res. **8**: 2453–2460.
39. **O'Brien, S. J.** 1984. Genetic maps. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
40. **Orr-Weaver, T. L., J. W. Szostak, and R. J. Rothstein.** 1983. Genetic applications of yeast transformation with linear and gapped plasmids. Methods Enzymol. **101**:228–245.
41. **Rigby, P. W. J., M. Dieckmann, C. Rhodes, and P. Berg.** 1977. Labeling deoxyribonucleic acid to high specific activity *in vitro* by nick translation with DNA polymerase I. J. Mol. Biol. **113**: 237–251.
42. **Rothstein, R. J.** 1983. One-step gene disruption in yeast. Methods Enzymol. **101**:202–211.
43. **Sandmeyer, S. B., and M. V. Olson.** 1982. Insertion of a repetitive element at the same position in the 5'-flanking regions of two dissimilar yeast tRNA genes. Proc. Natl. Acad. Sci. USA **79**:7674–7678.
44. **Schwartz, D. C., and C. R. Cantor.** 1984. Separation of yeast chromosome-sized DNAs by pulsed field gradient gel electrophoresis. Cell **37**:67–75.
45. **Schweizer, E., C. MacKechnie, and H. O. Halvorson.** 1969. The redundancy of ribosomal and transfer RNA genes in *Saccharomyces cerevisiae*. J. Mol. Biol. **40**:261–277.
46. **Sherman, F., G. R. Fink, and J. B. Hicks.** 1982. Methods in yeast genetics: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
47. **Southern, E. M.** 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98**:503–517.
48. **Stinchcomb, D. T., C. Mann, and R. W. Davis.** 1982. Centrometric DNA from *Saccharomyces cerevisiae*. J. Mol. Biol. **158**:157–179.
49. **Strathern, J. N., C. S. Newlon, I. Herskowitz, and J. B. Hicks.** 1979. Isolation of a circular derivative of yeast chromosome III: implications for the mechanism of mating type interconversion. Cell **18**:309–319.
50. **Struhl, K., and R. W. Davis.** 1981. Transcription of the *his3* region in *Saccharomyces cerevisiae*. J. Mol. Biol. **152**:535–552.
51. **Struhl, K., D. T. Stinchcomb, S. Scherer, and R. W. Davis.** 1979. High-frequency transformation of yeast: autonomous replication of hybrid DNA molecules. Proc. Natl. Acad. Sci. USA **76**:1035–1039.
52. **Studier, F. W.** 1969. The genetics and physiology of phage T7. Virology **39**:562–574.
53. **Szostak, J. W., and E. H. Blackburn.** 1982. Cloning yeast telomeres on linear plasmid vectors. Cell **29**:245–255.
54. **Winston, F., K. J. Durbin, and G. R. Fink.** 1984. The *SPT3* gene is required for normal transcription of TY elements in *S. cerevisiae*. Cell **39**:675–682.
55. **Yen, P. H., A. Sodja, M. Cohen, Jr., S. E. Conrad, M. Wu, N. Davidson, and C. Ilgen.** 1977. Sequence arrangement of tRNA genes on a fragment of *Drosophila melanogaster* DNA cloned in *E. coli*. Cell **11**:763–777.