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Physical Analysis of the *CYC1-sup4* Interval in *Saccharomyces cerevisiae*

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CYC1 and *sup4* are part of a tightly linked cluster of genes on chromosome X in the yeast *Saccharomyces cerevisiae*. Using as probes previously cloned fragments containing the *CYC1* and *sup4* genes, we have identified and cloned the deoxyribonucleic acid (DNA) present between these genes in one strain of yeast. We find that the *CYC1* and *sup4* genes are approximately 21 kilobases apart. In the same strain, the meiotic map distance is approximately 3.7 centimorgans, for a ratio of 5.6 kilobases per centimorgan in this interval. The physical mapping has allowed unambiguous determination of the orientation of *CYC1* and *sup4* relative to each other, the centromere, and a nearby transfer ribonucleic acid ($tRNA_2^{Ser}$) gene. The spontaneous mutation *cyc1-1* inactivates the *CYC1* gene as well as the neighboring loci *OSM1* and *RAD7*. We have determined that a *cyc1-1*-bearing strain lacks approximately 13 kilobases of single-copy DNA from the *CYC1-sup4* region, including all of the *CYC1* coding information. There is a sequence homologous to the middle-repetitive element Ty1 at or near the breakpoint of the *cyc1-1* deletion. We discuss the possibility that Ty elements play a role in the formation of such large, spontaneous deletions, which occur frequently in this region of chromosome X in certain yeast strains.

Recent technological advances have made it possible to isolate and purify specific segments of eucaryotic genomes by cloning them in *Escherichia coli*. We have used these techniques to study a region on the right arm of chromosome X in yeast, containing at least five closely linked genes: *CYC1, OSM1, RAD7, sup4,* and *CDC8* (10, 29). This region is one of the most intensely studied portions of the yeast genome. Detailed fine-structure genetic maps have been constructed at the *CYC1* and *sup4* loci (8, 21, 26). Lawrence et al. (10) have performed extensive tetrad analyses of strains marked at four of the loci in the cluster and at the flanking markers *ILV3, MET3,* and *CDC11*. A wide variety of deletions have been obtained in this region (21, 25, 29), including an intriguing class of events that are mediated by the specific mutator *DEL1* (11). The region near the *sup4* gene has been found to contain copies of two different "dispersed repetitive" elements, Ty1 and δ, which are present in many copies scattered throughout the genome of yeast at chromosomal sites which vary from one strain to another, and whose genetic role is presently unknown (3). For all of these reasons, we set out to obtain recombinant deoxyribonucleic acid (DNA) clones corresponding to the entire region and to study their physical arrangement. Extension of these studies to the *CYC1-sup4* DNA of various mutants and natural variants would allow a correlation between genetic phenomena, described at the level of phenotype, and the underlying DNA configuration. In addition, it would pave the way to an understanding of the physical organization of a group of linked genes.

The *CYC1* and *sup4* loci have been previously cloned in this laboratory (13, 17). In this paper we report the cloning of 38 kilobases (kb) of DNA which includes all of the material between the *CYC1* and *sup4* genes, as well as some flanking sequences. We have used the cloned DNA to make preliminary studies of two genetically observable phenomena of the region: recombination and deletion. The results illustrate the potential uses of this region in developing a better understanding of the DNA basis for genetic phenomena.

MATERIALS AND METHODS

Yeast strains and genetic methods. Strains D311-3A (*CYC1*$^+$), B596 (*cyc-91*, = *cyc1-9*), and D234-4D (*cyc1-1*) were originally obtained from Fred Sherman (26, 27). J12-2C and other strains bearing ochre mutations and ochre suppressors were obtained from Janet Kurjan; these strains are segregants of strain W87 or its mutant derivatives (22). S288C was originally from R. Mortimer.

Standard yeast genetic methods were used, as de-

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scribed by Mortimer and Hawthorne (14). Random spores were obtained by digesting asci with glusulase (Endo), blending briefly in a Vortex mixer, and then plating on enriched medium. Individual colonies were tested for haploidy by mating ability; those which could not mate were assumed to be diploid and were eliminated. The *CYC1* gene was scored by ability to grow on lactate as the sole carbon source (26). *SUP4* was scored by suppression of ochre mutations. Since *cyc1* mutants are difficult to score by conventional replica plating, cell suspensions were placed in microtiter wells and replicated onto plates by means of a replicator consisting of an array of steel bolts, by the method of Sherman et al. (25). Segregants which were particularly difficult to score were mated to a *cyc1-1* tester and scored as diploids for growth on lactate. The *cyc1-91* mutation is an ochre mutation, but it is very poorly suppressed by known ochre suppressors. Our *cyc1-91 SUP4-o* double mutant strain grew nearly as poorly on lactate as did its parent *cyc1-91 sup4*$^+$ strain.

Preparation of DNA. High-molecular-weight yeast DNA was prepared as described previously (18). Bacteriophage were grown lytically on L plates. For quick λ DNA preparations, the contents of one lysed plate were prepared by the procedure of Blattner et al (1). Larger-scale preparations of λ DNA were purified by the method of Thomas and Davis (32). Plasmid DNA was prepared by the procedure of Elwell et al. (5). For quick screening of recombinant plasmid clones, the following procedure was employed. Individual cultures of *E. coli* strains containing various recombinant plasmid clones were grown overnight in 10 ml of L broth. The cells were washed once in TE (10 mM tris(hydroxymethyl)aminomethane [Tris], pH 7.5–1 mM ethylenediaminetetraacetate (EDTA) and then suspended in 0.5 ml of cold 18% sucrose–35 mM Tris (pH 8)–0.1 M EDTA. Next, 0.065 ml of lysozyme (10 mg/ml) was added to each sample, and then the samples were placed on ice for 10 min. While the samples were still on ice, 0.5 ml of cold 1% Triton X-100–15 mM EDTA–50 mM Tris (pH 8.5) was added to each sample, and the cells were left on ice for an additional 10 min with occasional shaking. The samples were then placed at 80°C for 5 min, cooled on ice, then spun in a microfuge (Eppendorf centrifuge 5412) for 15 to 25 min. The clear supernatant, containing plasmid DNA, was poured off into a fresh tube. To each tube was added 2.5 μl of diethylpyrocarbonate; the mixtures were heated at 65°C for 15 min and then spun in the microfuge for 3 min. The supernatants were transferred to fresh tubes, ethanol precipitated twice, and then redissolved in 0.1 ml of TE. From 1 to 10 μl of each such sample was cleaved with 2 to 3 U of restriction enzyme and analyzed by agarose gel electrophoresis.

Enzymes and radioisotopes. Restriction endonucleases *Eco*RI, *Hin*dIII, *Sal*I, *Xba*I, *Bam*HI, and *Pst*I were obtained variously from Bethesda Research Laboratories, Inc., Miles Laboratories, Inc., New England Biolabs, and Boehringer Mannheim Corp. Cleavage reactions were carried out according to the conditions recommended by the enzyme supplier. DNA polymerase was purchased from Boehringer Mannheim, T4 DNA ligase was from Bethesda Research Laboratories, and α-^{32}P-labeled deoxycytidine triphosphate was from New England Nuclear Corp.

Manipulations of DNA. Gel electrophoresis, cloning and λ and plasmids, nick translation of DNA, and hybridization to Southern blots were all performed as described previously (13). The recombinant λ and plasmid clones used in this study are described in Tables 1 and 2. All organisms harboring recombinant DNA molecules were handled in a P2 facility, in accordance with the guidelines of the National Institutes of Health.

RESULTS

Cloning the *CYC1-sup4* interval. At the outset, it seemed feasible to clone all of the DNA between the *CYC1* and *sup4* genes, because these genes are closely linked genetically (10), and also because both genes had already been cloned on nonidentical *Eco*RI fragments from the same yeast strain (13, 17).

Preliminary experiments in which DNA from the *CYC1* and *sup4* clones was hybridized to yeast DNA that had been cleaved with various restriction enzymes established that the *CYC1* and *sup4 Eco*RI fragments were not only nonidentical but also nonadjacent in the genome. Consequently, we decided to search for chromosomal DNA sequences that adjoined the *CYC1* and *sup4 Eco*RI fragments, by constructing hybridization probes from the ends of each

TABLE 1. *Recombinant λ clones used in this work*

Recombinant λ chromosome	Vector	Cloned yeast restriction fragments[a]	Reference
λ-YeB	λgt *lac*	II, III, IV	13
λCSI-II	λ590	I, II	This work
λCSIV-VI	λ590	IV, V, VI	This work
λCSVI-IX	Charon 4	VI, VII, VIII, IX	This work
λgt5·Sc505[b]	λgt5	X, XI, XII	17
λCSXII-XIII	λ590	XII, XIII	This work
λYe *cyc1-1*	λgt1	II plus deletion breakpoint region from D234-4D (*cyc1-1*)	This work

[a] Roman numeral designations refer to Fig. 1. Yeast strain B596 was the source of DNA for cloning, except where noted.

[b] This clone has also been called λgt-ScB1 or B1 by Cameron et al. (3).

TABLE 2. *Recombinant plasmid subclones[a] used in this work*

Recombinant plasmid	Cloned yeast restriction fragments[b]	Parental λ clone[c]	Reference
pCS-II	II	λ-YeB	This work
pCS-III(pYe *cyc1-9*)	III	λ-YeB	13
pCS-IV	IV	λ-YeB	This work
pCS-IV-VI	IV, V, VI	λCSIV-VI	This work
pCS-VI	VI	λCSIV-VI	This work
pCS-VII	VII	λCSVI-IX	This work
pCS-VIII	VIII	λCSVI-IX	This work
pCS-IX	IX	λCSVI-IX	This work
pCS-X	X	λgt5·Sc505	This work
pCS-XI(pYT-A)	XI	λgt5·Sc505	6
pCS-XII	XII	λgt5·Sc505	This work
pYe *cyc1-1* (7.0)	Deletion breakpoint from D234-4D (*cyc1-1*)	λYe *cyc1-1*	This work

[a] In all cases, plasmid pBR322 was used as the vector, grown in *E. coli* strain RR1.
[b] Roman numeral designations refer to Fig. 1.
[c] All yeast DNA used was originally from strain B596, except where noted.

fragment and using these probes to screen a pool of *Hin*dIII fragments of B596 DNA cloned in the vector λ590 (16; Fig. 1A, lines 1, 2, and 3.) This type of strategy, called "overlap hybridization" (4) or "chromosome walking," has previously been applied to regions in yeast, *Drosophila melanogaster,* and chicken genomes (4, 23; W. Bender, P. Spierer, and D. S. Hogness, J. Supramolec. Struct. 3[Suppl.]:32, 1979).

The first round of overlap hybridizations yielded additional *CYC1*- and *sup4*-related sequences, but did not result in linking up the two groups of DNA clones (Fig. 1A, line 3). For further work, we concentrated on one direction only, namely, rightward off the 3′ end of the *CYC1* gene. There were two reasons for this choice. First, Lawrence et al. (10) had obtained genetic evidence which suggested that the orientation of the *CYC1* gene might be centromere–5′ *CYC1* 3′–*sup4*, implying that by "walking" from the end of the *CYC1* clone that was distal to the 3′ end of the *CYC1* gene one should eventually reach the *sup4* gene. The second reason for concentrating our attention on DNA sequences off the 3′ end of the *CYC1* gene was that during the time of these studies, we were also studying the physical nature of the *cyc1-1* mutation (see below). Genetically, this mutation deletes *CYC1* and *RAD7,* which are clustered with *sup4* in the order *CYC1–RAD7–sup4* (10), whereas *sup4* itself is not deleted (29). Physically, we found that one endpoint of the deletion was approximately 2.5 kb leftward from the 5′ end of the *CYC1* gene; the other endpoint extended rightward beyond our first round of clones. For this reason, as well, we chose to concentrate on further cloning in that direction.

This strategy proved successful. The next clone (Fig. 1A, line 5) contained the remaining DNA between the *CYC1* and *sup4* genes. Ultimately, each of the cloned DNA fragments was transferred from the λ vector in which it was initially isolated from the yeast chromosome to the plasmid vector pBR322 (Tables 1 and 2). The pBR 322 clones serve as our working stock of *CYC1-sup4* DNA for use as hybridization probes, for restriction mapping, and for other studies.

The results of the cloning have allowed unambiguous determination of the relative orientations of *CYC1* and *sup4,* as well as a nearby transfer ribonucleic acid ($tRNA_2^{Ser}$) gene whose presence on the same *Eco*RI fragment as *CYC1* was recently detected (G. S. Page, Ph.D. thesis, University of Washington, Seattle, 1978) (Fig. 1B). The orientation of the *CYC1* gene agrees with that suggested by Lawrence et al. (10) on genetic grounds. It is important to note that all of the cloning was done with DNA from a single yeast strain, B596. Physical distances between *CYC1* and *sup4,* as well as the locations of restriction sites, vary from one yeast strain to another in this interval. For example, strain D311-3A, the parent strain from which B596 was obtained by ultraviolet mutagenesis (26), possesses an *Eco*RI site in region III which strain B596 lacks (13). The single-base change which creates the *cyc1-91* mutation in strain B596 also happens to inactivate an *Eco*RI site within the *CYC1* gene.

A more significant polymorphism lies in the *sup4* end of the interval. Strain B596 contains a copy of Ty1, a dispersed repetitive element, approximately 2 kb from the 3′ end of the *sup4* gene, whereas strain S288c lacks Ty1 in that location (3). Strain W87 (22) and its derivatives, including J12-2C (8), have a configuration similar or identical to that of S288c (18; unpublished data). Restriction mapping and fragment sizing of regions VIII, IX, and X from strains B596,

S288c, and J12-2C indicate that there is little difference between the *CYC1-sup4* regions of these strains other than an insertion of Ty1 near *sup4* in B596 (Fig. 2 and 3). *CYC1* and *sup4* are 5.6 kb further apart in strain B596 than they are in strains S288C, W87, and J12-2C, due to the Ty1 insertion between *CYC1* and *sup4* in B596.

Genetic distance within the physically

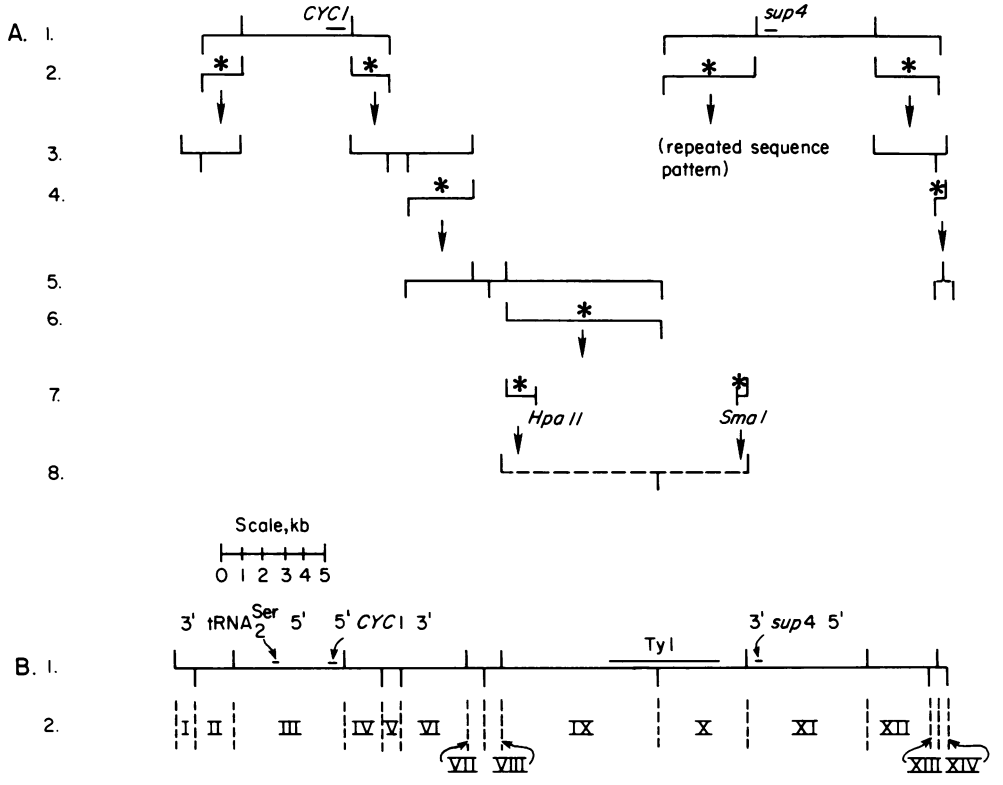

FIG. 1. *(A) The cloning of the CYC1-sup4 interval of strain B596. Clones are represented by solid horizontal lines; dashed horizontal lines indicate fragments identified by hybridization to Southern blots of agarose gels, but not cloned. Restriction sites are represented by vertical bars: HindIII sites by bars extending above the horizontal, EcoRI sites by bars extending below, and other sites as bars which extend in both directions. Line 1: The primary clones, containing the CYC1 and sup4 genes. Line 2: Subclones, labeled with* ^{32}P *by nick translation (indicated by asterisk), used to identify and clone overlapping fragments. Line 3: The fragments cloned in the first round of overlap hybridization screening. The vector used was λ590 (16). The left-hand subclone of the sup4-containing fragment contained part of a copy of a repeated sequence element, Ty1 (see text), which hybridized strongly to many restriction fragments of the yeast genome. Hence, this subclone, as such, could not be used to identify an overlapping fragment; see lines 7 and 8. Lines 4 and 5: The second round of overlap hybridization. The DNA shown in line 5 proved to be the final link between CYC1 and sup4. The vector used was Charon 4 (2), into which size-selected 10- to 25-kb fragments from a partial EcoRI digest of B596 DNA were inserted. Line 6: A subclone of the clone shown in line 5 was nick translated and hybridized to a Southern blot of EcoRI-cut yeast DNA, revealing the multiple-band pattern typical of the small EcoRI fragment of Ty1 (3). Line 7: Confirmation of the final overlap. Single-copy probes were derived from one end of each clone containing Ty1, as shown. Both probes hybridized to HindIII fragments of B596 of the same size, which is also the size predicted on the hypothesis that the two probes are on adjacent EcoRI fragments in B596. (See Fig. 2 for autoradiographic evidence.) Line 8: The 12-kb HindIII fragment, which constituted the final overlap. This fragment could not be cloned from our pool, since its size exceeded the capacity of the vector used. (B) Physical map of CYC1-sup4 interval of strain B596, derived from the clones in (A). Line 1: Restriction map of the interval, with HindIII and EcoRI restriction sites are indicated as in (A). The approximate location and size of each known genetic entity in the region are shown. Gene orientations are from DNA sequence analysis (6, 13; G. S. Page, Ph.D. thesis). Since the gene order is centromere-CYC1-sup4 (10), the centromere is to the left in this figure. Line 2: For purposes of discussion, the region has been divided into 14 intervals, with EcoRI and HindIII sites as landmarks. Intervals II through XII have been subcloned in plasmid pBR322.*

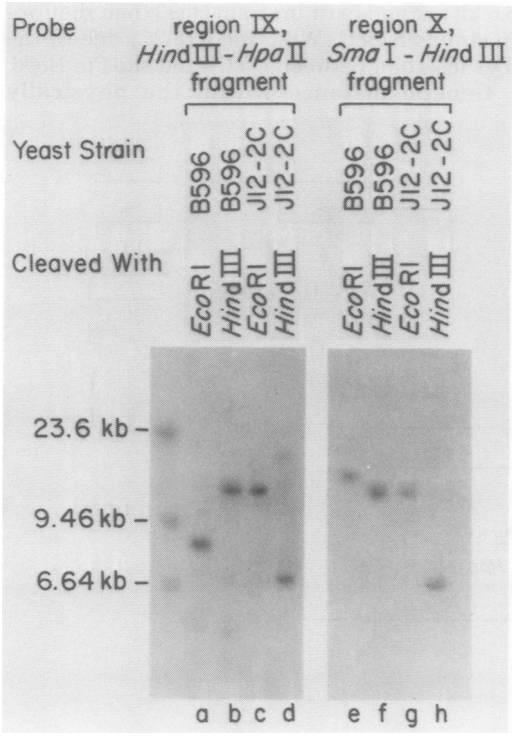

FIG. 2. *Confirmation of the final overlap and comparison of two strains which differ by the presence or absence of a Ty1 element near sup4. DNA of strain B596 (lanes a, b, e, and f) and strain J12-2C (lanes c, d, g, h) was cut with either EcoRI or HindIII as indicated and then separated by size on an agarose gel and blotted onto nitrocellulose. DNA of bacteriophage lambda was cut with HindIII and used as a size marker, using the sizes determined by P. Philippsen and R. Davis (personal communication). The blot was hybridized with ^{32}P-labeled DNA from regions IX or X, as indicated, as well as with lambda DNA to label the size marker fragments. For confirmation of the final overlap, note that both probes hybridized to HindIII fragment of B596 DNA (lanes b and f) of the same size, but the probes hybridized to differently sized EcoRI fragments of DNA of this strain (lanes a and e). The results provide the basis for the map in Fig. 1. For comparison of the B596- and S288c-type DNA configurations, note that in strain J12-2C (S288c-type), both probes hybridize to the same-size EcoRI fragment (lanes c and g) and HindIII fragment (lanes d and h), but in strain B596, the probes hybridize to different EcoRI fragments (lanes a and e). This is because a Ty1 element, which contains an EcoRI site, is situated between the two probed sequences in strain B596, but not in J12-2C. See Fig. 3 for a diagrammatic interpretation of these data.*

defined interval. The *CYC1-sup4* region affords the opportunity to compare the physical versus the genetic size of a DNA segment. The genetic distance between *CYC1* and *sup4* has been measured previously as 0.2 to 3 centimorgans (cM) in several crosses (10). Since the genetic size of a given interval in yeast varies from strain to strain (28), we decided to measure the genetic distance between *CYC1* and *sup4* in a diploid in which both copies of chromosome X shared a B596-type structure within this region, so that we could compare physical and genetic distances in the same strain. The construction of such strains is outlined in Fig. 4. Briefly, the rationale was to obtain a diploid whose *CYC1-sup4* DNA was physically homozygous, including the Ty1 insertion near *sup4* between heterozygous markers at both the *CYC1* and *sup4* loci. The physical homozygosity of the *CYC1-sup4* region of our diploid, PS25, was then confirmed by Southern analysis of restriction enzyme digests of its DNA, probing for regions III, VIII, IX, and X. The diploid was sporulated, and recombination between *CYC1* and *sup4* was measured by analyses of tetrads and random spores.

The results of the genetic analysis are shown in Table 3; 273 random spores and 89 tetrads were analyzed. For purposes of comparison, tetrads were converted to a "per spore" basis; however, this obscures the fact that the random spores represent 273 quasi-independent events, whereas the tetrads represent only 89 independent events. Some authors, for example, Lawrence et al. (10), exclude convertant tetrads from their determinations of genetic map distance; however, this tends to lower map distances as compared with determinations from random spores or nontetrad organisms, in which every recombinant counts as such, regardless of the type of meiosis from whence it came.

It can be seen from Table 3 that the ratio of physical to genetic distance in the *CYC1-sup4* interval of strain PS25 is somewhere between 4.0 and 6.6 kb/cM. Since each tetrad or random spore is statistically equivalent to one meiotic

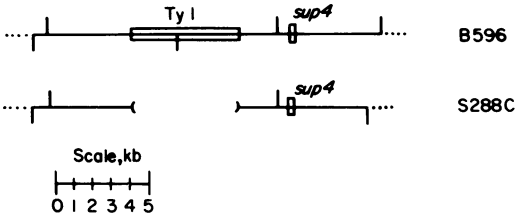

FIG. 3. *Polymorphism for the Ty1 near sup4. HindIII sites are represented by vertical lines extending above the horizontal line, EcoRI sites below. B596 lacks the rightmost EcoRI site found in S288c in this region, but that appears to be due to a small number of base changes rather than a gross difference between the strains (3). Note that the presence of Ty1 introduced an additional EcoRI site in the interval.*

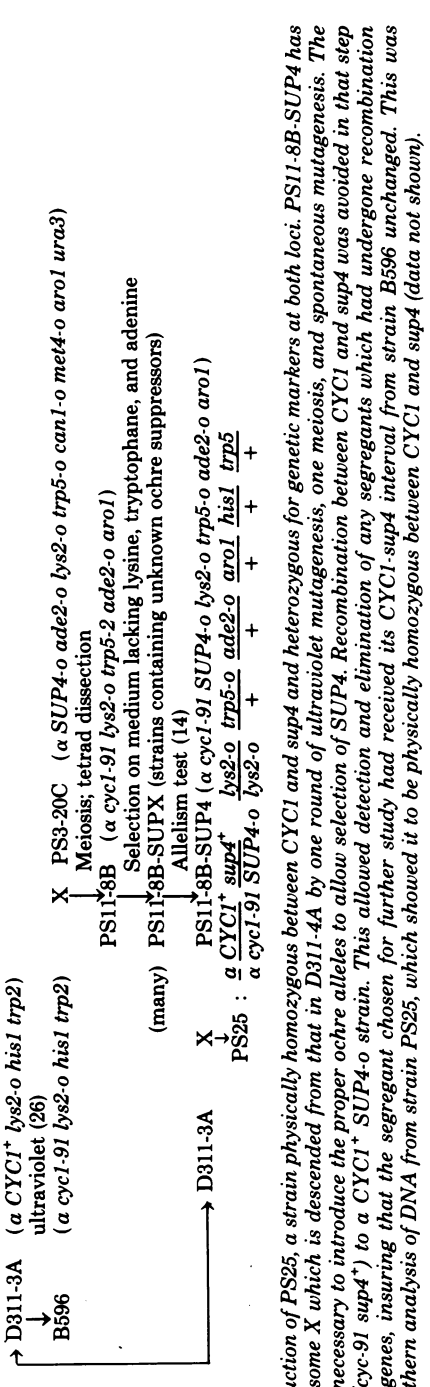

FIG. 4. *Construction of PS25, a strain physically homozygous between CYC1 and sup4 and heterozygous for genetic markers at both loci. PS11-8B-SUP4 has a copy of chromosome X which is descended from that in D311-4A by one round of ultraviolet mutagenesis, one meiosis, and spontaneous mutagenesis. The meiosis step was necessary to introduce the proper ochre alleles to allow selection of SUP4. Recombination between CYC1 and sup4 was avoided in that step by mating B596 (cyc-91 sup4+) to a CYC1+ SUP4-o strain. This allowed detection and elimination of any segregants which had undergone recombination between the two genes, insuring that the segregant chosen for further study had received its CYC1-sup4 interval from strain B596 unchanged. This was confirmed by Southern analysis of DNA from strain PS25, which showed it to be physically homozygous between CYC1 and sup4 (data not shown).*

TABLE 3. *Linkage of CYC1 and sup4 in segregants from PS25*

Tetrads or spores	Recombinant spores[a]	Parental spores[b]	Recombination (cM)	kb/cM
Segregated tetrads[c]	12	324	3.6	5.8
All tetrads[d]	19	337	5.3	4.0
Random spores	9	264	3.2	6.6

[a] *CYC1+ SUP4-o* or *cyc1-91 sup4+*.
[b] *CYC1+ sup4+* or *cyc1-91 SUP4-o*.
[c] Tetrads exhibiting 2:2 segregation at both loci (6 tetratypes, 78 parental ditypes).
[d] Five tetrads exhibited gene conversion, as follows: three *CYC+* to *cyc*, one *cyc* to *CYC+*, one *SUP4* to *sup4+*. One of the *CYC*-to-*cyc* tetrads also had a pair of reciprocal recombinant spores.

event, the best estimate is a weighted average, in which each random spore is given the same weight as each tetrad. Such an average comes out to 3.7 cM, or 5.6 kb/cM.

Estimates for the overall size of the yeast genome are 3,500 cM (15) and 1.4×10^4 kb (9). Based on these figures, the average ratio of physical to genetic distance in the yeast genome should be about 4 kb/cM. From studies of a large portion of chromosome III in yeast, Strathern et al. (31) found a ratio of 2.7 kb/cM, close to the estimated overall genome average. Our value for the *CYC1-sup4* interval is close to these estimates and is the first case, to our knowledge, in which the physical and genetic sizes of an interval have been measured in the same yeast strain.

Physical analysis of the *cyc1-1* mutation. We have used our clones as probes to study the *CYC1-sup4*-related DNA of a strain with an interesting lesion in the region. Strain D234-4D harbors a spontaneous mutation, *cyc1-1,* which behaves genetically as a deletion of all of the *CYC1* locus and also inactivates the neighboring loci *OSM1* and *RAD7,* but not *sup4* (29). We have obtained evidence that the *cyc1-1* mutation is a physical deletion, and that there is a repetitive DNA element at or near the site of deletion.

To determine whether *cyc1-1* is a physical deletion of *CYC1*-related DNA, DNA was prepared from strain D234-4D, cleaved with *Eco*RI or *Hin*dIII (or both), separated on agarose gels, blotted onto nitrocellulose filters, and then hybridized with various probes cloned from the *CYC1-sup4* region of strain B596. Probes including all of regions IV through VIII, as well as the right-hand 2.4-kb portion of region III and the left-hand 2.5-kb portion of region IX, failed to hybridize to DNA from the deletion strain, indicating that these single-copy sequences are absent from that strain. Regions III and X do contain single-copy sequences which are present

in the deletion strain (Fig. 5). Hence, one endpoint of the deletion is within the area corresponding to region III, and the other is within the area corresponding to region IX or X. Further Southern analysis, using region IX subfragments as probes, revealed that all of the single-copy DNA of region IX, except for the left-hand 2.5 kb, hybridizes to *cyc1-1* DNA. Hence, the right-hand endpoint of the *cyc1-1* deletion is in region IX. All in all, approximately 13.5 kb of single-copy DNA found in the *CYC1-sup4* region of strain B596 is absent from the genome of the *cyc1-1* strain.

A dispersed repetitive element similar to Ty1 (3) is present in the deletion strain at or near the site of the missing 13 kb of single-copy DNA. This conclusion is based on the analysis of a cloned *Eco*RI fragment of *cyc1-1* DNA which was obtained by using region III as a probe. The cloned *cyc1-1* fragment contained some sequences homologous to region III, up to the beginning of the region of deleted DNA, but also contained sequences homologous to Ty1. The presence of Ty1 sequences on the *cyc1-1* clone was revealed when the clone was radioactively labeled and hybridized to Southern blots of *Eco*RI-cut yeast DNA. Rather than hybridizing to one or a few bands, the clone hybridized strongly to many bands, in a pattern typical of the "large half" of Ty1, the same half that is found in region X of strain B596 (Fig. 6). (Ty1 has a single internal *Eco*RI site, which divides the element into a large half and a small half. Each half gives a different, diagnostic multiple-band when hybridized to Southern blots of *Eco*RI-cut yeast DNA.)

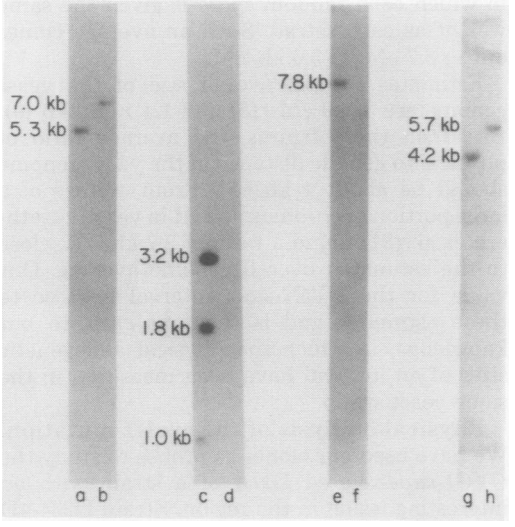

FIG. 5. *Comparison of DNA of strain B596 (cyc1-91) and strain D234-4D (cyc1-1 deletion) in the CYC1-sup4 region. DNA of each strain was doubly cut with EcoRI and HindIII, run on an agarose gel, blotted onto nitrocellulose, and then hybridized with various probes from strain B596. Lanes a, c, e, and g: B596. Lanes b, d, f, and h: D234-4D. Lanes a and b: The region III probe hybridized to a band in both strains, but the bands were of different sizes. Lanes c and d: The region IV, V, and VI probe hybridized to three fragments of B596 DNA, and did not hybridize to any DNA of D234-4D. Similar results are obtained with probes for regions VII and VIII (data not shown). Lanes e and f: The left-hand 1.5-kb HindIII-HpaII fragment of region IX hybridized to the appropriate agment of B596 DNA, and not at all to DNA of strain D234-4D. Lanes g and h: The right-hand 0.4-kb SmaI-HindIII fragment of region X hybridized to DNA of both strains, though to fragments of different zes. Therefore, the cyc1-1 deletion extends to a point somewhere in regions IX and X.*

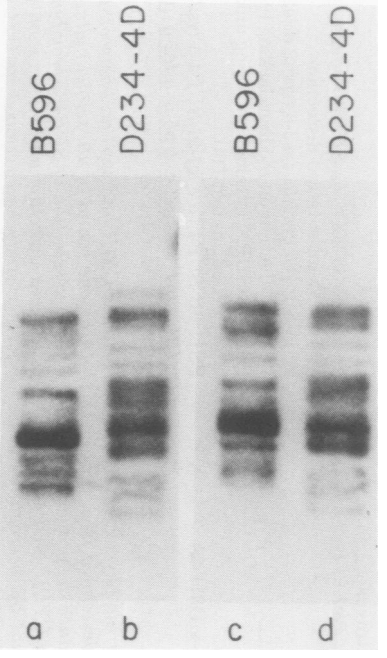

FIG. 6. *Demonstration that a Ty1-like element is present at or near the breakpoint of the cyc1-1 deletion. DNA of strains B596 (lanes a and c) and D234-4D (lanes b and d) was cleaved with EcoRI, separated by size on an agarose gel, blotted onto nitrocellulose, then hybridized with ^{32}P-labeled DNA clones. The probe for lanes a and b was the region X clone, which contains the large EcoRI fragment of Ty1 (3). For lanes c and d, the probe was pYecyc1-1, the cloned fragment containing the breakpoint of the cyc1-1 deletion from strain D234-4D. Both probes gave nearly the same, characteristic strain-specific mainfold of multiple bands.*

DISCUSSION

The data presented above illustrate the potential of the *CYC1-sup4* region for studying various genetic phenomena at the DNA level. The genetic and physical information thus far obtained indicates that there are at least seven genetic entities in this region of chromosome X: $tRNA_2^{Ser}$, *CYC1, OSM1, RAD7,* Ty1, *sup4,* and *CDC8.* Some of these (i.e., *OSM1, RAD7,* and *CDC8*) have not been placed on the physical map. In addition, other genes, not yet identified by mutation, may be found in the area covered by the available clones. RNA-DNA hybridization experiments have identified a transcript which maps to a portion of the DNA between the $tRNA_2^{Ser}$ gene and the *CYC1* gene (D. Leung, personal communication; G. Faye, personal communication). In all likelihood there are still other unidentified transcribed sequences in the 15.5 kb of single-copy DNA between *CYC1* and *sup4.* These can be identified by physical mapping of RNA transcripts to cloned DNA fragments. The function of such "genes" is unknown, and most of them must be dispensable, since a substantial amount of the DNA in question can be deleted without cell death or gross phenotypic abnormality.

Once additional genetic markers are placed on the physical map, this region recommends itself as a system for studying the relationship between physical distances and recombination frequencies as well as the effects of physical heterozygosities and unlinked modifiers on recombination. Such studies require that physically characterized, congenic strains with the appropriate genetic markers be constructed. Since transformation allows the construction of congenic yeast strains, differing only in the interval of choice (24), such experiments are now feasible.

We are particularly interested in the process of deletion formation in the *CYC1-sup4* region. Liebman et al. (11) have described a strain-specific activity, called *DEL1,* which acts as a mutator in this region. Strains carrying *DEL1* give rise spontaneously, at high frequency, to mutations which are genetically indistinguishable from the *cyc1-1* deletion described above. *DEL1* is dominant in both *cis* and *trans* configurations, is present in certain laboratory strains of yeast but not others, and is closely linked to the *CYC1-sup4* interval on chromosome X. The *cyc1-1* mutation, a spontaneous mutation of unknown origin, is suspected to have arisen from such a *DEL1*-bearing strain (11).

The presence of a Ty1-like element at or near the breakpoint of the *cyc1-1* deletion leads us to suspect that the element may have been involved in the formation of the deletion. Bacterial translocatable elements, which share some features with Ty1 (7), are known to promote deletions in their vicinity (e.g., reference 20). In bacteria, deletions induced by translocatable elements often have one breakpoint at the end of the element and the other breakpoint in neighboring DNA. The *cyc1-1* deletion appears to have such a structure. Roeder and Fink (19) report that a Ty1-like element inserted near the *HIS4* gene can promote deletions and other DNA rearrangements.

Further experiments on the physical structure of the *CYC1-sup4* interval can be directed toward an understanding of the relationship between Ty1-like elements and deletions in the region. The mere presence of a Ty1 element between *CYC1* and *sup4* does not produce a high frequency of deletions of *CYC1*; strain D311-3A (equivalent to B596) has a Ty1 element between the two genes, yet it is not a "deletor" strain. If Ty1 or related elements can indeed induce deletions in the *CYC1-sup4* region, we must conclude that the Ty1 near *SUP4* in strain D311-3A is not competent to induce *cyc1-1*-type deletions, because of either its location, orientation, or internal structure.

Perhaps Ty1-like elements must flank a region to delete it. A study of spontaneous deletions of *sup4* and surrounding sequences (21) suggests that those deletions are mediated by small repetitive elements, called δ, which flank the *sup4* gene. We have recently found a laboratory strain, similar to the *DEL1* strains of Liebman et al. (11), which produces numbers of spontaneous deletions which are genetically and physically indistinguishable from *cyc1-1.* We are now in the process of analyzing the *CYC1-sup4* DNA of this deletor strain and deletions derived from it, as well as Liebman's *DEL1* strain and deletions derived from it. Preliminary results indicate that the region to be deleted is flanked by Ty1-like elements in the parent strains.

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