JOURNAL OF BACTERIOLOGY, Apr. 1978, p. 237-245
0021-9193/78/0134-0237$02.00/0

Vol. 134, No. 1
Printed in U.S.A.

Ribosomal DNA Magnification in *Saccharomyces cerevisiae*

DAVID B. KABACK† AND HARLYN O. HALVORSON*

Department of Biology, Rosenstiel Basic Medical Sciences Research Center, Brandeis University, Waltham, Massachusetts 02154

Received for publication 16 September 1977

Strains monosomic for chromosome I of *Saccharomyces cerevisiae* contain 25 to 35% fewer rRNA genes than do normal diploid strains. When these strains are repeatedly subcultured, colonies are isolated that have magnified their number of rRNA genes to the diploid amount while remaining monosomic for chromosome I. We have determined the amount of DNA complementary to rRNA in viable haploid spores derived from a magnified monosomic strain. Some of these haploids contained 24 to 48% more rRNA genes than a normal euploid strain. These extra genes may be responsible for the increased number of rRNA genes in the strain monosomic for chromosome I. Genetic analysis of the haploids containing extra rRNA genes suggested that these genes are linked to chromosomal DNA and are heterozygous. They were not closely linked to any centromere and were not located on chromosome I. Furthermore, all the DNA complementary to rRNA in one of these haploid strains with magnified rRNA genes sedimented at a chromosomal molecular weight, consistent with chromosomal linkage. In addition, several new mutations mapping on chromosome I were used to show that ribosomal DNA magnification was not due to a chromosome I duplication.

The haploid genome of *Saccharomyces cerevisiae* contains approximately 140 genes for rRNA (24). These genes are tandemly repeated (4, 5), and 50 to 70% of them are located on chromosome I (8, 14, 16, 19). The location of the remaining genes is unknown, although the available evidence suggests that these genes are also chromosomal (3, 8). Strains monosomic ($2n - 1$) for chromosome I initially contain approximately 25% fewer genes coding for rRNA than do diploid strains. Upon continued subculturing, colonies are found containing the same amount of ribosomal DNA (rDNA) as that found in diploid strains while remaining monosomic for chromosome I (15, 16). The increase in the number of genes coding for rRNA in yeast may be analogous to the magnification of the rRNA genes in *Drosophila melanogaster bobbed* mutants (1, 22).

To arrive at a mechanism for rDNA magnification in yeast, it is necessary to determine the arrangement of the additional rDNA. In this paper we report a genetic analysis of rDNA in the magnified monosomic strain. We found that this strain contains some additional rDNA not present in the unmagnified strain and that the additional rDNA is transmitted to haploid spores during meiosis. The observed pattern of inheritance of the rDNA suggests that the rDNA is chromosomal. In addition, in the haploid strain tested, all rDNA sediments with intact chromosomes in sucrose gradients, supporting the idea that the magnified rDNA is chromosomal. Finally, we were unable to find any evidence that magnification was caused by a duplication of part of chromosome I translocated to another chromosome.

† Present Address: Division of Chemistry and Chemical Engineering, California Institute of Technology, Pasadena, CA 91125.

MATERIALS AND METHODS

Media. Cells were grown on YEPD liquid (1.0% [wt/vol] yeast extract [Difco Laboratories]-2.0% [wt/vol] peptone [Difco]-2.0% [wt/vol] dextrose). For agar plates, 2.0% (wt/vol) Difco agar was added to the above.

Genetics. Genetic manipulations were as described previously (17, 18, 25). The strains used are listed in Table 1.

Isolation of ts mutations mapping on chromosome I. A 3-day stationary-phase culture of a strain monosomic for chromosome I (X1221a-7c) was mutagenized by treatment with 2.0% (wt/vol) ethyl methane sulfonate for 60 to 80 min (13). This treatment did not lead to a detectable loss of viability. The cells were directly plated on YEPD agar at a density of 200 cells per plate and incubated at 25°C for 2 days. The plates, now containing colonies approximately 2 mm in diameter, were replica plated to prewarmed YEPD agar and incubated 1 day at 37°C. Colonies unable to grow at 37°C were scored and then picked from the plates grown at 25°C. Approximately 10^5 colonies were screened, yielding 5 thermosensitive (ts) mutants that were able to sporulate, giving viable haploid progeny.

TABLE 1. *Strains*

Strain	Genotype	Source
X1221a-7c 10/29	a *ade1* *arg4* *trp1* *leu1* *his*x[a] *met1* *ade*x[b] *thr1* mag rDNA[c] α 0 + + + + + + +	
X1221a-7c silica 1	a *ade1* *arg4* *trp1* *leu1* *his*x[a] *met1* *ade*x[b] *thr1* α 0 + + + + + + +	J. Bruenn
DK8	a *ade1* *arg4* *trp1* *leu1* α *ade1* + + +	Constructed from two haploids from X1221a-7c silica 1
X1221a-7c 10/29 1a	a *ade1* *leu1* *his*x[a]	
X1221a-7c 10/29 1b	α *ade1* *ade*x[b] *arg4* *leu1* mag rDNA	
X1221a-7c 10/29 2a	a *ade1* *ade*x[b] *trp1* *arg4* *leu1* mag rDNA	
X1221a-7c 10/29 2b	α *ade1* *trp1* *arg4* *leu1*	
DK64	a *ade1* *leu1* *his*x[a] + + α *ade1* *leu1* + *arg4* *ade*x[b]	Cross between X1221-7c 10/29 1a and 1b
DK65	a *ade1* *trp1* *arg4* *leu1* + α *ade1* *trp1* *arg4* *leu1* *ade*x[b]	Cross between X1221-7c 10/29 2a and 2b
DK17	a *tse* *ade1* + + α + + *ura1* *trp5*	
DK19	a *tsh* *ade1* + *leu2* + *lys1* α + + *ura1* + *trp5* +	
X3402-15c	a $\frac{ADE1}{ADE1}$ *his5* *ura3* *leu1* *arg1* *met2* *arg4* *gal1* *pet17* *ade5*	R. K. Mortimer
NHK678	a *cys1*	S. Fogel
D649	a *MAL2* *trp1* *pet6* *ade2* *lys2* *his4* *leu2* *thr4* *mal* *ade1* α + + + + + + + + + +	F. Sherman

[a] *his2* or *his8*.
[b] *ade6* or *ade9*.
[c] mag rDNA, 24 to 48% more rDNA than normal.

Haploid segregants were obtained by dissection of asci. Poor spore viability (in some cases less than 10%) was observed. All haploid segregants used contained recessive ts mutations that segregated 2:2, wild type/mutant.

Meiotic mapping. Since initial spore viability of the mutagenized monosomes was poor, haploid thermosensitive mutants were crossed to healthy haploids and dissected several times in succession until a diploid giving high spore viability was obtained. Linkage of the *ade1* gene to the ts mutation was determined by tetrad analysis (25).

Mitotic mapping. Mitotic crossing-over on chromosome I can be monitored by picking red-sectored colonies from *ade1*/+ heterozygotes. The red sector is due to homozygosis of the *ade1* gene when there is a crossover between the chromosome I centromere and the *ade1* gene. If the ts mutation is distal to the *ade1* gene on the same arm of chromosome I, a single crossover between the centromere and *ade1* gene results in a red sector that is frequently thermosensitive due to the cosegregation of the ts mutation. Diploids were constructed by crossing *ade1* ts haploids to wild-type haploids of opposite mating type. Approximately 2,000 cells were plated on YEPD agar at a density of 200 cells per plate. Mitotic recombination was induced by treatment with a 253-nm UV light for 20 s at 12 ergs/mm per s. Viability was not affected by this treatment. After 2 to 3 days of growth at 25°C, red sectors were picked. Purified red colonies from these sectors were tested for thermosensitivity on YEPD plates at 37°C.

Disome exclusion mapping (trisomic analysis). Crosses between thermosensitive mutants and a strain

disomic for chromosome I will yield tetrads that are 4:0, 3:1, and 2:2 (+:ts) if the ts mutation maps on chromosome I (18). X3402-15c, a strain disomic for chromosome I, was crossed to ts *ade1* double mutants and monitored for both aberrant *ade1* and ts segregation.

[^{3}H]DNA isolation. Cells grown to early stationary phase in YEPD liquid containing 0.5 μCi of [*G*-^{3}H]-adenine per ml (New England Nuclear Corp.) were broken with an Eaton press (7), and the DNA was extracted as previously described (16).

[^{32}P]rRNA isolation. ^{32}P-labeled 18S and 25S rRNA was isolated from strain D649 as previously described (23).

DNA-RNA hybridization. Filter hybridizations were performed at saturating conditions (24) by a modification of the procedure of Gillespie and Spiegelman (9), as previously described (15, 16). A minimum of three filters per DNA preparation were incubated in the same vial at a given rRNA concentration. Only differences greater than 11% of the control were considered significant. This conclusion is based on variations observed with different DNA preparations of the same strain.

Hybridization of rRNA to DNA sedimented in sucrose gradients. DNA from lysed spheroplasts was sedimented in 5 to 25% sucrose gradients according to the method of Blamire et al. (2). Fifty-milliliter gradients were centrifuged for 16 to 17 h at 11,000 rpm in an SW 25.2 rotor (Beckman Instruments). One-milliliter fractions were collected, heated for 10 min at 100°C, and quenched in ice. One milliliter of 4× SSC (0.6 M NaCl, plus 0.06 M sodium citrate, pH 7.0) was added to each fraction. DNA was fixed to filters, and hybridizations were performed as described previously (16). Hybridizations were done at an rRNA concentration of 3 μg/ml in a sealed sterile 125-ml Erlenmeyer flask containing 50 ml of 2× SSC-0.2% (wt/vol) sodium dodecyl sulfate.

RESULTS

Meiotic segregation of rDNA in the magnified monosomic strain. The magnified monosomic strain (X1221a-7c 10/29) was sporulated on potassium acetate plates (25) and dissected, and the haploid spores were cultured on YEPD. Monosomic strains produce two dead spores (no chromosome I [15]) and two viable ones. The amount of DNA complementary to 18S and 25S rRNA was determined for DNA isolated from the two viable haploid segregants from each of nine asci. DNAs isolated from a related diploid (DK8) and a magnified monosomic strain (X1221a-7C 10/29) were used as controls. These two strains contain the euploid level of rDNA (15).

The surviving spore pairs from the magnified strain fell into two classes (Table 2). In the first class (tetrads no. 1, 2, 3, 8, 11, 12, and 13) the DNA from one spore hybridized 24 to 48% more rRNA than did the other spore and the controls. In the other class, DNA isolated from the two viable spores (tetrads no. 4 and 10) hybridized about the same amount of rRNA as did the controls. It is unlikely that the results reflect variable amounts of DNA per cell, since the magnified and unmagnified monosomic strains and DK8 all have the same (±9%) amount of DNA per cell and genetic analysis has not revealed any large degree of aneuploidy in the monosomic strains. It is also doubtful that there are large differences in the amount of mitochondrial DNA per cell, which could affect our results since the maximum variations observed (10) were insufficient to cause a 24 to 48% difference in hybridization.

If the additional rDNA in spores from X1221a-7c 10/29 represents rRNA genes involved in magnification, spores from the unmagnified monosomic strain should contain euploid rDNA amounts. This possibility was tested by determining the amount of DNA complementary to 18S and 25S rRNA in DNA isolated from the viable haploids from three tetrads of the unmagnified monosome X1221a-7c silica 1. We found (Table 3), with one exception, that all spores tested contain the euploid amount of rDNA. It was not apparent why spore 2a hybridized slightly less rRNA. These results indicate that the occurrence of extra rDNA in haploids is a feature of only magnified strains.

The pattern of segregation of extra rDNA in X1221a-7c 10/29 haploids suggests the presence of one or more heterozygous chromosomal clusters of rDNA genes that are not linked to chromosome I. In seven out of nine tetrads, the extra rDNA appeared to segregate at the second meiotic division with respect to both the lethality caused by chromosome I monosomy and a heterozygous *trp1* mutation (both *trp1* and viability segregate exclusively at the first meiotic division). This suggests that the extra rDNA is not tightly linked to a centromere.

A heterozygous cluster of rRNA genes in the magnified monosomic strain should also give a third class of tetrad, where both spores contain extra rDNA. The failure to observe this class may be expected, considering the limited number of tetrads examined. A cluster of rRNA genes not linked to a centromere would give a tetrad ratio of 1:1:4 (one tetrad containing two unmagnified spores:one tetrad containing two magnified spores:four tetrads containing one magnified and one unmagnified spore) for magnified DNA. The ratio observed (0:2:7) is within the 95% probability limits (χ^2) for 1:1:4, consistent with a heterozygous non-centromere-linked cluster of genes.

To further test if magnified rDNA is behaving as an additional rRNA gene cluster, we examined whether extra rDNA segregates 2:2 in het-

TABLE 2. *Hybridization of 18S and 25S rRNA to haploids from the magnified monosome for chromosome I*[a]

Expt no.	Strain	Percent total DNA hybridized to rRNA at different [^{32}P]rRNA concn					
		2 μg/ml	3 μg/ml	4 μg/ml	6 μg/ml	Avg	Difference between spores in an ascus (%)
1[b]	X1221a-7c 10/29 1a	1.66	1.50	2.32		1.83 ± 0.43	42
	X1221a-7c 10/29 1b	2.50	2.25	2.99		2.58 ± 0.27	
	X1221a-7c 10/29 3a	2.36	2.54	3.24		2.71 ± 0.46	38
	X1221a-7c 10/29 3b	1.48	2.10	2.30		1.96 ± 0.43	
	X1221-7c 10/29	2.06	1.95	2.54		2.18 ± 0.31	
	DK8	1.59	2.19	1.92		1.90 ± 0.30	
1a	X1221a-7c 10/29 1b		2.94	3.05	2.76	2.93 ± 0.10	
2[b]	X1221a-7c 10/29 2a	2.73		2.81	3.06	2.87 ± 0.17	48
	X1221a-7c 10/29 2b	1.76		1.92	2.15	1.94 ± 0.20	
	X1221a-7c 10/29 4a	1.65		2.03	1.88	1.85 ± 0.19	10
	X1221a-7c 10/29 4b	1.49		2.38	2.26	2.04 ± 48.0	
	X1221a-7c 10/29	1.99		2.46	2.08	2.18 ± 0.25	
	DK8			2.10	2.30	2.21 ± 0.14	
3	X1221a-7c 10/29 8a		2.46	2.28	2.68	2.47 ± 0.20	30
	X1221a-7c 10/29 8b			3.26	3.14	3.20 ± 0.08	
	DK8		2.36	2.49	2.73	2.53 ± 0.19	
4	X1221a-7c 10/29 10a		2.14	2.20	2.38	2.24 ± 0.12	10
	X1221a-7c 10/29 10b		1.93	2.04	2.14	2.04 ± 0.11	
	X1221a-7c 10/29 11a		2.33	2.20	2.44	2.32 ± 0.12	35
	X1221a-7c 10/29 11b		3.11	3.09	3.22	3.14 ± 0.07	
	X1221a-7c 10/29 12a		2.84	3.01	2.89	2.91 ± 0.09	24
	X1221a-7c 10/29 12b		2.24	2.40	2.42	2.35 ± 0.10	
	X1221a-7c 10/29 13a		1.85	2.23	2.04	2.04 ± 0.19	37
	X1221a-7c 10/29 13b		2.58	2.79	3.00	2.79 ± 0.21	
	DK8		2.24	2.23	2.17	2.20 ± 0.04	
	DK8		2.14	2.18	2.29	2.20 ± 0.08	

[a] [^{32}P]rRNA was hybridized to [^{3}H]DNA fixed to nitrocellulose filters as described in the text. The percent DNA hybridized at each concentration is the average of three separate filters. Separate experiments are grouped with their respective controls. All filters from a single experiment were incubated in the same vial for a given rRNA concentration.

[b] In experiments 1 and 2 it appears that conditions were not saturating at 2 μg of rRNA per ml, which may account for the low plateau values observed.

TABLE 3. *Hybridization of 18S and 25S rRNA to haploids from the unmagnified monosome for chromosome I*[a]

Strain	Percent total DNA hybridized to rRNA at different [^{32}P]rRNA concn			
	4 μg/ml	6 μg/ml	Avg	Difference between spores in ascus (%)
X1221a-7c silica 1 1a	2.48	2.01	2.25 ± 0.24	10
X1221a-7c silica 1 1b	2.11	1.97	2.04 ± 0.18	
X1221a-7c silica 1 2a	1.74	1.95	1.85 ± 0.29	20
X1221a-7c silica 1 2b	2.17	2.28	2.22 ± 0.33	
X1221a-7c silica 1 3a	2.30	2.17	2.24 ± 0.33	1
X1221a-7c silica 1 3b	2.36	2.16	2.26 ± 0.24	

[a] [^{32}P]rRNA was hybridized to [^{3}H]DNA fixed to nitrocellulose filters as described in the text. The percent DNA hybridized at each rRNA concentration is the average of three separate filters.

erozygotes. DK64, a diploid heterozygous for extra rDNA, was sporulated and dissected, and the amount of rDNA was determined in four cultured haploids from one tetrad. DNA from DK8 was used as a control. The results (Table 4) do not give a straightforward 2:2 segregation for extra rDNA, as would be predicted for a single heterozygous cluster of genes. Although two spores (2a and 2d) contained roughly the same amount (5 and 11% more, respectively) of rDNA as the control, spores 2b and 2c gave rDNA values significantly higher than the control, yet not equal to each other. A similar pattern was observed in a preliminary experiment on a tetrad from DK65 (another strain heterozygous for extra rDNA), where two spores contained the normal rDNA level, whereas the other spores contained 50 and 30% more, respectively, than the two spores with the wild-type rDNA level (D. Alexandraki and D. B. Kaback, unpublished data). We suggest that these results may be caused by unequal meiotic crossing-over between sister chromatids carrying extra rDNA. Subsequent segregation of the sister strands would result in the observed unequal amounts of extra rDNA in spores 2b and 2c.

The amount of DNA complementary to 18S and 25S rRNA was also measured in DK64 and DK65. The results (Table 4) indicate the presence of magnified rDNA in the heterozygous strains. If the magnified rDNA behaves as a stable part of a normal chromosome, the amount of DNA complementary to rRNA in DK64 and DK65 should be intermediate between the levels of rDNA in the parent haploids. Magnified haploids contain 24 to 48% more rDNA than do unmagnified haploids; therefore, a heterozygote should contain 12 to 24% more rDNA than normal. Although the results for DK64 and DK65 appear 10% too high to be intermediate (35 and 27% higher, respectively, than DK8), this may reflect the low value obtained for DK8 in this experiment. Comparison of DK64 and DK65 with the previously published value for DK8 (2.24%) gives 24 and 17%, respectively, more rDNA in the heterozygotes: values within the intermediate range.

TABLE 4. *Segregation of magnified rDNA in a diploid*[a]

Strain	Percent total DNA hybridized to rRNA at different [^{32}P]rRNA concn			
	3 μg/ml	5 μg/ml	6 μg/ml	Avg
DK64-2a	1.97	2.15	2.18	2.15 ± 0.15
DK64-2b	3.22	3.24	3.31	3.26 ± 0.05
DK64-2c	2.54	2.56	2.76	2.62 ± 0.20
DK64-2d	2.20	2.44	2.33	2.32 ± 0.14
DK8	1.88	2.02	2.18	2.06 ± 0.22
DK64	2.68	2.66	3.04	2.79 ± 0.22
DK65	2.51	2.61	2.75	2.62 ± 0.10

[a] [^{32}P]rRNA was hybridized at saturating conditions to [^{3}H]DNA fixed to nitrocellulose filters as described in the text. [^{3}H]DNA was isolated from two diploids constructed by crossing a magnified haploid to an unmagnified haploid (DK64 and DK65), from diploid control (DK8) and from four haploids of a DK64 tetrad. The percent DNA hybridized at each rRNA concentration is the average of three separate filters.

Sedimentation of magnified rDNA in sucrose gradients. The normal complement of rDNA in yeast sediments with intact chromosomal DNA molecules on a sucrose gradient (8). To test the chromosomal origin for the magnified rDNA, we examined the distribution of DNA complementary to rRNA in sucrose gradients containing intact chromosome-size DNA molecules. Figure 1a shows the results of such an experiment, using a magnified haploid segregant of X1221a-7c 10/29. The two controls for this experiment were an unmagnified haploid segregant of X1221a-7c 10/29 (Fig. 1b) and DK8 (Fig. 1c).

The sedimentation of [^{3}H]DNA in each gradient resembled the profiles observed by Finkelstein et al. (8) and Petes and Fangman (20), with the center of the main peak sedimenting at approximately 115S. In the three strains tested, over 90% of the DNA hybridizing to rRNA sedimented at a faster rate than did the T4 DNA marker, indicating that most, if not all, of the rDNA was of chromosomal molecular weight. The [^{32}P]rRNA bound to filters with material from the bottoms of the gradients (approximately 10 to 20% of the total ^{32}P counts) was assumed to be due to adsorption of rRNA to cellular debris. Quantitation of the percent DNA hybridized across the gradient (fractions 2 to 40) confirmed the increased rDNA content in the magnified haploid (D. Kaback, unpublished observation), indicating that the solubilized DNA (fractions 2 to 40) was representative of the total DNA loaded on a gradient. Finkelstein et al. (8) observed that DK8 contained a broad distribution of rDNA with a peak on the light side of the main DNA peak. Our results show that in all strains examined most of the rDNA is evenly distributed over the entire DNA peak, with an enrichment for approximately 25% of the total rDNA on the heavy side of the main DNA peak. In addition, the magnified haploid showed a small rDNA peak on the light side of the main DNA peak, which was reduced or missing completely in the controls. This difference could account for only a small fraction of the additional DNA in X1221a-7c 10/29 1b. We were

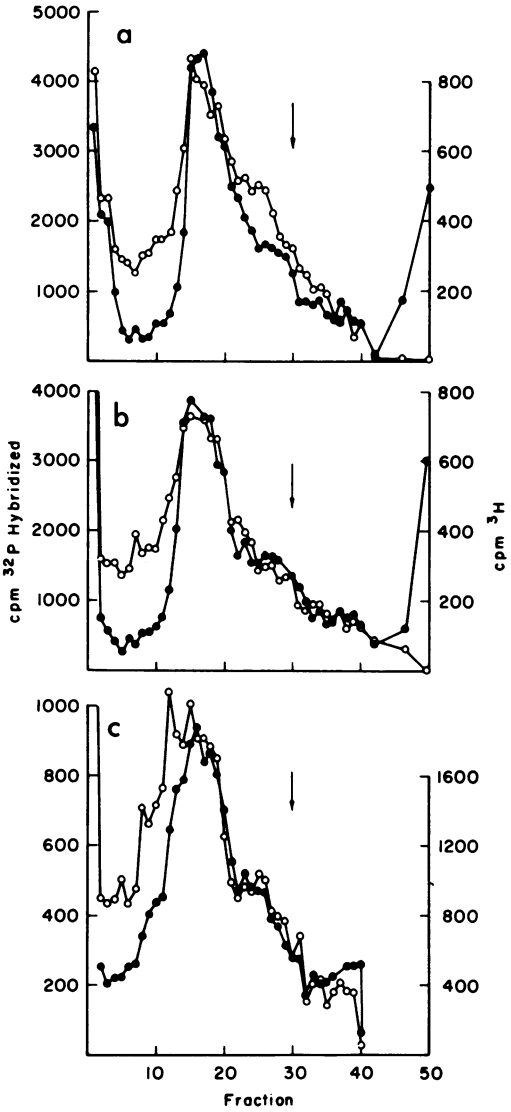

FIG. 1. *Sedimentation of sequences complementary to [^{32}P]rRNA in sucrose gradients of chromosome-size DNA molecules. Approximately 5 × 10^7 exponential-phase cells from haploids derived from the magnified monosome and the related diploid were continuously labeled with [^{3}H]adenine (10 μCi/ml) in YEPD, converted to spheroplasts, lysed, and layered on top of gradients (2). The gradients were centrifuged at 11,000 rpm for 16 to 17 h and fractionated, and the amount of rDNA in each fraction was determined by hybridizing ^{32}P-labeled 18S and 25S rRNA at saturating conditions to denatured DNA fixed to nitrocellulose filters. Sedimentation is from right to left. Arrow represents sedimentation of intact [^{3}H]thymidine-labeled bacteriophage T4 (kindly provided by Edward Goldberg). Symbols: ●, [H^3]DNA; ○, [^{32}P]rRNA hybridized. (a) X1221a-7c 10/29 1b, magnified haploid. (b) X1221a-7c 10/29 1a, unmagnified haploid. (c) DK8, control diploid; DK8 was examined by using [^{32}P]rRNA with a lower specific activity, accounting for the fewer counts hybridized; in addition, fractions 41 to 50 were not assayed for DK8.*

unable to repeat the previously observed (8) bimodal distribution of rDNA. On the basis of our experiments, we conclude that most, if not all, of the rDNA in the magnified haploid is of chromosomal molecular weight, suggesting that the magnified rDNA has a chromosomal origin. If the magnified rDNA is extrachromosomal, it must be very large, at least the size of an intact chromosome, or have a stable association with chromosomal DNA.

Is rDNA magnification due to a translocation? One mechanism by which magnified rDNA could arise is by a duplication of the chromosome I rRNA genes becoming translocated onto another chromosome. Besides chromosome I, some rDNA may be located on at least one other chromosome (3, 8, 14, 16, 19). If rDNA is located on other chromosomes, a translocation could occur by mitotic crossing-over between homologous rDNA regions on chromosome I and another rDNA-containing chromosome. It has recently been suggested that all rDNA in normal strains is located on just one chromosome (21). In this case, a genetic translocation of chromosome I material could still occur, but by some other mechanism. In either case, mitotic segregation of the translocation would result in a strain monosomic for chromosome I and heterozygous for an rDNA-containing chromosome I translocation on the other chromosome. If so, other genes from chromosome I might also be translocated with the rDNA, leading to duplication of these genes in the magnified monosomic strain. During meiosis, a translocation containing the magnified rDNA should segregate into the spores, giving haploids with elevated rDNA redundancies and duplications for the other translocated genes.

One difficulty in testing this hypothesis is that only a few genes have been identified on chromosome I. We, therefore, screened and isolated mutations on chromosome I (S. A. Henry, D. B. Kaback, and H. O. Halvorson, unpublished data). An ethyl methane sulfonate-mutagenized strain monosomic for chromosome I was screened for recessive ts mutations. Five mutants were initially isolated that grow on YEPD agar at 25 but not at 37°C. Mitotic mapping (Table 5a) was used to show that two of the five mutations, *tse* and *tsh*, map to the right of *ade1* on the right arm of chromosome I. Meiotic link-

TABLE 5. *Genetic analysis of thermosensitive mutants mapping on chromosome I*[a]

a. Mitotic segregation: concomitant segregation of ts with ade1

Strain	Genotype	Red sectors tested	Thermosensitive red sectors
DK 17	$\frac{ade1\ tse}{+\ +}$	17	5
DK 19	$\frac{ade1\ tsh}{+\ +}$	19	7

b. Meiotic linkage of ts to ade1

Type	*tse*	*tsh*
Parental ditype	6	17
Nonparental ditype	3	3
Tetratype	20	53

c. Disome exclusion

Cross	*ade1*, no. of asci segregating			*ts*, no. of asci segregating		
	4:0	3:1	2:2	4:0	3:1	2:2
DK17-2c *ade1 tse* × X3402-15c disome I	4	0	2	2	2	2
DK19-1b *ade1 tsh* × X3402-15c disome I	4	0	0	2	2	0

[a] Linkage of *tse* and *tsh* to chromosome I was confirmed by three independent methods. Detailed descriptions of these types of analyses are found in reference 18.

age between *ade1* and the two ts mutations was also apparent from the large proportion of parental ditype asci compared to nonparental ditype asci (Table 5b). Quantitation of the tetrad data places *tse* and *tsh* 66 and 49 centimorgans from *ade1*, respectively. *tse* and *tsh* neither complement nor recombine with other (Klar and Kaback, unpublished data) and are therefore assumed to be allelic. At present, it is not clear why the map distances to *ade1* differ. Perhaps these aberrant results are due to the small tetrad sample size or to a heavily mutagenized genetic background in one of the strains. In either case, more data are required to firmly establish the genetic map positions of these mutations.

The linkage of *tse* and *tsh* was confirmed by disome exclusion analysis (Table 5c). Strains trisomic for chromosome I were constructed by crossing both *tse* and *tsh* haploids to a chromosome I disome. The strains were sporulated, dissected, and scored for *ade1*. The crosses involving both *tse* and *tsh* gave 4:0, 3:1, and 2:2 (ts:mutant) tetrads for ts and *ade1*, confirming their linkage to chromosome I.

The magnified haploids were tested for duplications of both the *tse* and the *tsh* markers and for the *cys1* marker (11), a mutation on the left arm of chromosome I. It was unneccessary to test the *ade1* gene, since we had previously shown (15) that it is not duplicated in the magnified monosomic strain. Both a magnified haploid, X1221a-7c 10/29 1b, and an unmagnified haploid, X1221a-7c 10/29 1a, were crossed to either *cys1*, *tse*, or *tsh* haploids. The diploids were sporulated, and at least 12 complete tetrads from each cross were analyzed for segregation of the chromosome I markers. In both strains tested, *cys1*, *tse*, and *tsh* gave only 2:2 (+:−) segregation, demonstrating that none of these genes were duplicated in the magnified rDNA-containing haploids. This result indicates that magnification did not occur as a result of a translocation for the parts of chromosome I tested. Since the exact position of the rDNA on chromosome I is not known, it is possible that we may have been testing parts of chromosome I not closely linked to the rDNA. Therefore, the present results do not completely exclude translocation as a possible mechanism for magnification.

DISCUSSION

An investigation of the genetic organization of the magnified rDNA in *S. cerevisiae* led to the following conclusions. Magnified rDNA is transmitted into haploids during meiosis. This rDNA is localized neither on chromosome I nor on a trisomic chromosome, nor is it homozygous. The available evidence suggests that it may be arranged in one or more heterozygous rRNA gene clusters localized on very few chromosomes.

Inheritance of magnified rDNA. To elucidate the mechanism for rDNA magnification, we have investigated the genetic arrangement of the magnified rDNA. We found that many haploids from the magnified monosome contain more rDNA than do haploids from the unmagnified monosome. Since magnification in the chromosome I monosome eliminates the rDNA deficiency, the amount of magnified rDNA in X1221a-7c 10/29 should be the same as the amount that normally maps on the chromosome I. The results show that the amount of extra rDNA segregating into haploids is not enough to account for all the magnified rDNA. An average of only 47 extra rRNA genes segregate into the magnified haploids (Table 6). Thus, at least 30 of the magnified rRNA genes either are unstable through meiosis or segregate into the nonviable spores. Therefore, our conclusions concerning the organization of the magnified rDNA are applicable only to the extra rDNA found in the viable haploids.

Magnified rDNA is neither on a trisomic chromosome nor on chromosome I, nor is it homozygous. Several arrangements for the magnified rDNA are incompatible with the tetrad analysis data on rRNA gene redundancy. The first possibility to be ruled out is the presence of aneuploidy for an rDNA-containing chromosome. The magnified strain is still monosomic for chromosome I (15), eliminating the possibility of an extra copy of chromosome I. Therefore, any extra chromosome would have to be a trisome for another rDNA-containing chromosome. During meiosis, a trisomic chromosome could segregate at first division with chromosome I and at second division segregate into the two viable spores, giving two disomes containing more rDNA than wild-type haploids. Alternatively, a trisomic chromosome could segregate away from the monosomic chromosome I into the two nonviable spores. The viable spores in this case would both contain the wild-type level of rDNA. In both cases, the two viable spores from a single ascus would contain the same amount of rDNA, and this was not observed.

A second arrangement that we consider unlikely is that the extra rDNA is all linked to the monosomic copy of chromosome I. If this were the case, the extra rDNA would segregate with chromosome I into all the viable spores. All the viable spores would then contain more rDNA than the wild type, which was also not observed.

Finally, if the extra rDNA were present at the same site in equal amounts on two homologous chromosomes, we would expect all the viable spores to contain equal additional amounts of rDNA. The elevated rDNA redundancy in this case would be less than predicted if the additional rDNA were all on chromosome I, since the extra rDNA would be divided on two homologous chromosomes instead of one. The nonequivalence of the rDNA levels in the haploids from the magnified monosome also rules out this arrangement.

TABLE 6. *Number of rRNA genes involved in rDNA magnification*[a]

Ploidy	DNA hybridized (%)	No. of rRNA genes
$2n$	2.24	270
$2n - 1$	1.60	195
$2n - 1$ + mag rDNA	2.26	271
n + mag rDNA	2.95	177
n	2.17	130

[a] Values for n and n + mag rDNA are the average of all haploids determined to be in those classes. Values for $2n$ and $2n - 1$ are from Kaback et al. (14). All values are calculated on the basis of a haploid genome size of 1.2×10^{10} daltons (12) and a molecular weight for 18S and 25S rRNA of 2.0×10^6 (26).

Magnified rDNA is localized on very few genetic elements. If the magnified rDNA were dispersed on many DNA molecules, we would predict that these DNA molecules, if stable, would be randomly distributed into the spores during meiosis, giving most of the spores a slightly higher level of rDNA than normal. Since over half the spores from the magnified monosomic strains contain the normal level of rDNA and only large amounts of extra rDNA are found in the rest, we conclude that the additional rDNA is not widely dispersed but is located on only a few DNA molecules. However, it also appears likely that the magnified rDNA is arranged on more than one DNA molecule, because only some of the magnified rDNA present in the magnified monosomic strain is found segregating into the viable haploids. This suggests that the rest of the magnified rDNA is segregating on another DNA molecule into the nonviable spores. Furthermore, in the subsequent cross involving the magnified haploids, a strict 2:2 segregation for the magnified rDNA is not observed, suggesting that the magnified rDNA in the viable haploids may also be located on more than one DNA molecule, though it is also possible in this case that the difference in the amount of rDNA in the magnified haploids could be caused by unequal crossing over. The precise arrangement of the magnified rDNA must await further genetic characterization. Unfortunately, the ability to examine large numbers of tetrads is impaired by the laborious rDNA assay. In addition, magnified monosomic strains, as well as haploids derived from these strains, contain no obvious phenotype difference proportional to the rRNA gene redundancy, complicating the possibilities for a rapid genetic analysis.

Chromosomal linkage of magnified rDNA. The evidence that rDNA is incorporated into the duplex DNA structure of yeast chromosomes comes from the observations that yeast rDNA does not band as a dense satellite in equilibrium gradients using high-molecular-weight DNA (4, 6) and that monosomy for chromosome I is initially associated with a deficiency in rDNA (8, 14, 16, 19). The tetrad data for the magnified monosomic strain can be explained by a chromosomal linkage for the magnified rDNA. To obtain physical evidence for chromosomal linkage, intact chromosome-molecular-weight DNA was sedimented in sucrose gradients, and rDNA was found to move with the intact DNA molecules. However, it is still possible that the magnified rDNA is extrachromosomal and is

arranged in some manner on an episome of chromosomal size. A molecule of this nature could segregate into the haploid spores, though it might not behave like a normal chromosome during meiosis. Therefore, a direct physical or genetic test of chromosomal linkage for the magnified rDNA is still required.

rDNA magnification genetics. The observation that strains monosomic for chromosome I are 25 to 35% deficient for rDNA appears inconsistent with the report that all rDNA in normal strains is linked to a single chromosome (21). If all the rDNA is located on chromosome I, we would expect a strain monosomic for chromosome I to contain 50% less rDNA than does a diploid. One possible explanation for this apparent contradiction is that partial rDNA magnification had already occurred before the original monosomic strain (X1221a-7c silica 1) was tested. As mentioned previously, we do not think the magnified rDNA is located on chromosome I.

We have shown that the problem of rDNA magnification in yeast is amenable to genetic analysis. Initial results showed that rDNA magnification was not due to a chromosome I translocation. Further studies of this kind combined with physical and biochemical studies should help decide the number and location of the genetic elements involved in rDNA magnification, information that is essential for working out the details for a molecular mechanism.

ACKNOWLEDGMENTS

We thank Susan Henry for suggesting the use of the chromosome I monosome in screening for mutations mapping on chromosome I. We also thank Ann Sodja and John Fincham for helpful criticism.

This work was supported by a Public Health Service (PHS) grant from the National Institute of Allergy and Infectious Diseases (AI 10610). D.B.K. was a PHS trainee.

LITERATURE CITED

1. **Atwood, K. C.** 1968. Some aspects of the bobbed problem in *Drosophila*. Genetics **61**(suppl.):319–327.
2. **Blamire, J., D. R. Cryer, D. B. Finkelstein, and J. Marmur.** 1972. Sedimentation properties of yeast nuclear and mitochondrial DNA. J. Mol. Biol. **67**:11–24.
3. **Byers, B., and L. Goetsch.** 1975. Electron microscopic observations on the meiotic karyotype of diploid and tetraploid *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. U.S.A. **72**:5056–5060.
4. **Cramer, J. H., M. M. Bhargava, and H. O. Halvorson.** 1972. Isolation and characterization of rDNA of *Saccharomyces cerevisiae*. J. Mol. Biol. **71**:11–20.
5. **Cramer, J. H., F. W. Farrelly, and R. H. Rownd.** 1976. Restriction endonuclease analysis of ribosomal DNA from *Saccharomyces cerevisiae*. Mol. Gen. Genet. **148**:233–241.
6. **de Kloet, S. R.** 1973. Distribution of ribosomal ribonucleic acid cistrons among yeast chromosomes. J. Bacteriol. **114**:1034–1039.
7. **Eaton, N. R.** 1962. New press for disruption of microorganisms. J. Bacteriol. **83**:1359–1360.
8. **Finkelstein, D. B., J. Blamire, and J. Marmur.** 1972. Location of ribosomal RNA cistrons in yeast. Nature (London) New Biol. **240**:279–281.
9. **Gillespie, D., and S. Spiegelman.** 1965. A quantitative assay for DNA-RNA hybrids with DNA immobilized on a membrane. J. Mol. Biol. **12**:829–842.
10. **Hall, R. M., P. Nagley, and A. W. Linnane.** 1976. Biogenesis of mitochondria. XLII. Genetic analysis of the control of cellular mitochondrial DNA levels in *Saccharomyces cerevisiae*. Mol. Gen. Genet. **145**:169–175.
11. **Halos, S., and S. Fogel.** 1974. Cysteine mutants in *Saccharomyces cerevisiae*, p. 37. *In* Fourth International Symposium on Yeasts.
12. **Hartwell, L. H.** 1974. *Saccharomyces cerevisiae* cell cycle. Bacteriol. Rev. **38**:164–198.
13. **Lindegren, G., Y. L. Hwang, Y. Oshima, and C. C. Lindegren.** 1965. Genetical mutants induced by ethylmethanesulfonate in *Saccharomyces*. Can. J. Genet. Cytol. **7**:491–499.
14. **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.
15. **Kaback, D. B., and H. O. Halvorson.** 1977. Magnification of the genes coding for rRNA in *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. U.S.A. **74**:1177–1180.
16. **Kaback, D. B., H. O. Halvorson, and G. M. Rubin.** 1976. Location and magnification of 5S RNA genes in *Saccharomyces cerevisiae*. J. Mol. Biol. **107**:385–390.
17. **Mortimer, R. K., and D. C. Hawthorne.** 1969. Yeast genetics, p. 385–460. *In* A. H. Rose and J. S. Harrison (ed.), The yeasts. Academic Press Inc., New York.
18. **Mortimer, R. K., and D. C. Hawthorne.** 1973. Genetic mapping in *Saccharomyces*. Mapping of temperature-sensitive genes and use of disomic strains in localizing genes. Genetics **74**:33–54.
19. **Øyen, T. B.** 1973. Chromosome I as a possible site for some rRNA cistrons in *Saccharomyces cerevisiae*. FEBS Lett. **30**:53–56.
20. **Petes, T., and W. L. Fangman.** 1972. Sedimentation properties of yeast chromosomal DNA. Proc. Natl. Acad. Sci. U.S.A. **69**:1188–1191.
21. **Petes, T. D., and D. Botstein.** 1977. Simple Mendelian inheritance of the reiterated ribosomal DNA of yeast. Proc. Natl. Acad. Sci. U.S.A. **74**:5091–5095.
22. **Ritossa, F. M.** 1968. Unstable redundancy of genes for ribosomal RNA. Proc. Natl. Acad. Sci. U.S.A. **60**:509–516.
23. **Rubin, G. M.** 1975. Preparation of RNA and ribosomes from yeast, p. 45–64. *In* D. Prescott (ed.), Methods in cell biology, vol. 12. Academic Press Inc., New Yrok.
24. **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.
25. **Sherman, F., G. R. Fink, and C. W. Lawrence.** 1972. Methods in yeast genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
26. **Udem, S. A., and J. R. Warner.** 1972. Synthesis and processing of ribosomal RNA in *Saccharomyces cerevisiae*. J. Mol. Biol. **65**:227–242.