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Genetics

Magnification of genes coding for ribosomal RNA in *Saccharomyces cerevisiae*

(yeast/chromosome I monosome/DNA coding for rRNA)

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ABSTRACT **When a strain of *Saccharomyces cerevisiae* monosomic for chromosome I and initially deficient for 25% of the genes coding for ribosomal RNA is repeatedly subcultured, the number of these genes increases to and remains stable at the number in the wild type. This strain shows 2:2; viable: inviable first division segregation and hemizygosity for the *ade1* gene (a chromosome I marker), evidence that the strain is still monosomic for chromosome I. The increase in the number of genes coding for ribosomal RNA in yeast may be analogous to the magnification of the ribosomal RNA genes in *Drosophila melanogaster bobbed* mutants.**

Saccharomyces cerevisiae contains approximately 140 genes coding for rRNA per haploid genome (1). Approximately 60% of these genes are located on chromosome I (2–4) while the remainder are thought to be located on at least one but not more than two other chromosomes (5). The number of rRNA genes had appeared to be invarient because several unrelated euploid strains contain the same number of rRNA genes (1, 2, 4). Recently, a strain with an unusually high proportion of DNA coding for rRNA (rDNA) has been found (6); however, the nature of this increase has not been investigated.

Variations in the number of rRNA genes occur in *bobbed* mutants of *Drosophila melanogaster*. These mutants have been shown to be deficient in rDNA (7) and have a phenotype characterized by short bristle hairs and a thin cuticle (8). *Bobbed* mutants revert at a high frequency and show a concomitant increase in rDNA. Two types of reversion have been characterized. The first, termed rDNA compensation (9), is not a true genetic reversion because it is not inherited and appears to be limited to somatic tissues (9, 10). The second kind of reversion, termed rDNA magnification, is accompanied by a hereditable increase in the number of rRNA genes (9, 11). We have found that an event similar to rDNA magnification occurs in a strain of *S. cerevisiae* that is deficient in rDNA. Strains monosomic ($2n-1$) for chromosome I lack 25–35% of the genes coding for rRNA when compared to diploid strains (2–4, 21). After many transfers and many doublings the number of rRNA genes in a strain monosomic for chromosome I increases to the diploid level while remaining monosomic.

MATERIALS AND METHODS

Cell Growth. Cells were grown on YEPD liquid (1.0% yeast extract/2.0% Bactopeptone/2.0% dextrose) at 30°. Growth was monitored with a Klett-Summerson Photometer at 660 nm. Cells were harvested approximately 30 min after reaching stationary phase and washed with ice-cold distilled H_2O.

Isolation of [^{3}H]DNA and 18S and 26S [^{32}P]rRNA. These were carried out as described (12, 21).

Saturation DNA·RNA Filter Hybridization. Hybridization experiments were done in 0.3 M NaCl/0.03 M sodium citrate at pH 7.0 + 0.2% sodium dodecyl sulfate for 16 hr at 65° (21). At least three filters for a DNA sample were incubated at a given rRNA concentration. All filters for a given RNA concentration were incubated in a single vial.

Genetic Analysis was done as described (13).

DNA Determinations from Whole Cells. DNA was extracted from approximately 10^9 cells in logarithmic phase by a modification of the method described by Parry and Cox (14). Cells were washed twice with distilled water at 4°. The samples were then sequentially washed twice with both 10% trichloroacetic acid and 95% ethanol at 4°. DNA was extracted by treating the samples with 1 ml of 1.0 M perchloric acid at 70° for 20 min, centrifuging in a clinical centrifuge, and reextracting the pellet with 1 ml of 1.0 M perchloric acid at 70° for 20 min. The supernatants from both extractions were combined and recentrifuged to remove any particulate matter. Of a total 2.0 ml, 1.5 ml was assayed by a modification of the diphenylamine method (15) by adding an equal volume of the diphenylamine reagent [2.0 g of recrystallized diphenylamine in 100 ml of glacial acetic acid + 0.5 ml of 1:50 (vol:vol) solution of acetaldehyde] and mixing in a Vortex mixer for 30 sec. The samples were incubated for 18 hr at 30°, and the optical density was determined at 600 nm. Salmon sperm DNA was used as a standard. Cell number was determined by hemocytometer, counting large buds as separate cells.

RESULTS

Strains monosomic for chromosome I contain less DNA complementary to rRNA than diploid strains (2–4). We had been working with these strains in unrelated studies that required frequent transfers on YEPD agar. During this period all transferred single colonies were tested and confirmed monosomic by tetrad analysis. In a routine assay of rDNA levels a derivative of the original monosomic strain, X1221a-7c *ADE+* was found to contain the same relative amount of rDNA as a diploid strain (21). To determine whether or not this increase in rDNA was a rare, if not singular occurrence, we measured the amount of rDNA in a stock of the monosome for chromosome I, X1221a-7c 10/29, that had also been cultured over an extended period. We compared the amount of rDNA in X1221a-7c 10/29 with that of an isolate of the same strain that had not been extensively cultured, X1221a-7c silica 1, and with two related diploids, one that had been cultured, DK8 10/29, and one that had not, DK8 silica 1. Yeast have the advantage that they can be stored for long periods in the absence of growth by suspension in silica gel (16). The two isolates that had not undergone extensive growth were obtained from silica gel stocks made soon after the strains were either constructed in our laboratory or received from others. Table 1 summarizes the results of five separate experiments where the level of rDNA was

Abbreviation: rDNA, DNA coding for ribosomal RNA.

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Table 1. Increase in the number of genes coding for 18S and 26S RNA

Exp. no.	Strain	rRNA concentration (μg/ml) 2	3	4	5	6	Average
1	X1221a-7c 10/29			2.13	2.85	2.97	2.65 ± 0.83
	DK8 10/29			2.35	2.31	2.46	2.36 ± 0.15
	X1221a-7c silica 1			1.38	1.41	1.41	1.40 ± 0.04
	DK8 silica 1			2.47	2.47	2.15	2.37 ± 0.26
2	X1221a-7c 10/29	2.06	1.95	2.54			2.18 ± 0.31
	DK8 10/29	1.59	2.19	1.92			1.90 ± 0.30
3	X1221a-7c 10/29	1.99		2.46		2.08	2.19 ± 0.25
	DK8 10/29			2.10		2.32	2.21 ± 0.14
4	X1221a-7c 10/29			2.68	2.05	2.65	2.46 ± 0.50
	X1221a-7c silica 1			1.50	1.73	1.78	1.73 ± 0.04
				1.81	1.82	1.73	
5	X1221a-7c 10/29		2.34		2.31	2.41	2.35 ± 0.05
	X1221a-7c silica 1		1.63		1.79	1.70	
			1.81		1.71	1.87	1.76 ± 0.03
			1.86		1.66	1.80	
Average	X1221a-7c 10/29			2.32 ± 0.24			
	X1221a-7c silica 1			1.69 ± 0.16			
	DK8 10/29			2.16 ± 0.28			
	DK8 silica 1			2.37 ± 0.26			

rDNA levels were determined by saturation hybridization of 18S and 26S [^{32}P]rRNA to [^{3}H]DNA isolated from extensively cultured (10/29) and uncultured (silica 1) isolates of X1221a-7c, the monosome for chromosome I, and DK8, a related diploid. The results are expressed as the percent of total DNA hybridized.

measured in the above strains. The results of several experiments are shown because the values for a single experiment sometimes give standard deviations too large to permit an unambiguous determination of the percent DNA hybridized for a single DNA preparation. The results show that X1221a-7c 10/29 contains approximately the same amount of rDNA as both isolates of DK8, while X1221a-7c silica 1 contains approximately 25% less. The percent DNA hybridized in all strains is in close agreement with previously published results for diploid and monosomic strains (1–4). The level of rDNA in both diploids tested is approximately equal, suggesting that in euploid strains the number of rRNA genes remains constant irrespective of continued growth. Contrary to the diploid strains, the strain monosomic for chromosome I, initially 25% deficient in rRNA genes, now contains the diploid number of these genes.

Genetic analysis of monosomic strain

To determine if X1221a-7c 10/29 was identical to X1221a-7c silica 1, except for the increase in rDNA, we conducted a detailed genetic analysis of both strains. Tetrad analysis of all markers of X1221a-7c 10/29 and X1221a-7c silica 1 shows that the genotypes of both strains are identical (Table 2). In addition, both strains contain a heterozygous "lacy" colonial morphology mutation and another heterozygous adenine marker (either *ade6* or *ade9*), none of which was scored. Most important, the two isolates gave exclusively 2:2; viable:inviable first division segregation with respect to other centromere markers, indicating a monosomic chromosome. It should be noted that the strains showed a predominance of 2:0 *TRP 1:trp* 1 tetrads. Although we have no explanation for this aberrant behavior, we suspect it has no bearing on the increase in rDNA because both X1221a-7c 10/29 and X1221a-7c silica 1 show the same behavior.

It is possible to imagine that the increase in rDNA could have occurred by sporulation and subsequent mating of the spores, giving a diploid with two copies of chromosome I yet still containing some unknown heterozygous lethal mutation. We consider this extremely unlikely because, if these events were to occur, it would be expected that several heterozygous markers in the parent would become homozygous due to the random assortment of chromosomes during meiosis.

To show that both X1221a-7c 10/29 and X1221a-7c silica 1 are hemizygous for the *ade1* gene (a chromosome I centromere marker), as expected for strains monosomic for that chromosome, we performed the following experiment. If the *ade1* gene is hemizygous, then upon meiosis of a *ADE1+* revertant, all haploid spores would be *ADE1+* because each viable spore

Table 2. Tetrad analysis of strains monosomic for chromosome I (number of asci segregating)

Locus	X1221a-7c silica 1 2:0	1:1	0:2	X1221-7c 10/29 2:0	1:1	0:2
ade1	0	0	22	0	0	19
arg4	6	3	13	9	7	2
leu1	9	1	12	10	2	7
trp1	16	0	6	13	0	6
*his**	5	9	8	4	11	4
thr3†	4	5	7	2	3	0
met1†	6	9	2	1	3	1
M.T.‡	3a:a	9a:α	4α:α	1a:a	8a:α	4α:α

Asci were dissected and grown on YEPD agar. Viability was close to 50%, as expected for a monosomic strain. The two viable spores were replica-plated on synthetic media lacking the growth factors indicated. The numbers refer to the ratio of prototrophs to auxotrophs per ascus.

* *his2* or *his8*.

† Not all tetrads scored for *thr3* and *met1*.

‡ M.T., mating type.

Table 3. Determination of DNA per cell* in X1221a-7c 10/29, X1221a-7c silica 1, and the related diploid, DK8

	X1221a-7c 10/29	X1221a-7c silica 1	DK8
	2.16	2.14	2.45
	2.31	2.27	2.45
	2.53	2.18	2.28
	2.39	2.10	2.29
	2.39	2.06	2.38
	2.43	2.18	2.32
Average	2.37 ± 0.12	2.16 ± 0.06	2.36 ± 0.08

Cells growing exponentially were harvested and treated as described in *Materials and Methods*. DNA was determined by the diphenylamine reaction (15). Cell number was determined by hemocytometer. Buds at least ½ the size of the mother cells were included in the cell counts as a whole cell. Errors are the 95% confidence limits for the mean.

* Values are given as daltons of DNA per cell × 10^{-10}.

would contain the single copy of chromosome I present in the monosomic strain. Alternatively, if chromosome I is diploid, then *ADE1+* revertants would be heterozygous and would segregate both *ADE1+* and *ade1−* haploids. Five *ADE+* prototrophs from both X1221a-7c 10/29 and X1221a-7c silica 1 were isolated by plating approximately 5 × 10^8 cells on either complete medium minus adenine or minimal medium at a density of 5 × 10^7 cells per plate. Only large white colonies appearing after 3 days of growth at 30° were selected to minimize picking external suppressors of the *ade1* locus, which are often slow growing. The *ADE+* colonies were sporulated and dissected. In four out of five revertants of X1221a-7c silica 1, the rDNA-deficient strain, viable haploids were all *ADE1+*, as determined by complementation and/or absence of red pigmented (*ade1*) segregants. The fifth revertant segregated both *ADE+* and *ade−* haploids. It was suspected that this revertant was caused by an external suppressor because the *ade1* marker was segregating at the second meiotic division with respect to *trp1*, a trait not characteristic of the centromere-linked *ade1* gene. The presence of an unlinked suppressor of the *ade1* gene was confirmed in this revertant by crossing an *ADE+* segregant from the revertant with an unrelated *ADE1+* haploid of opposite mating type. The resultant diploid was sporulated and shown to still segregate *ade1* haploids, indicative of an external *ade1* suppressor. In all five *ADE+* revertants of X1221a-7c 10/29 (the strain that contained the diploid level of rDNA), all spores were shown to be *ADE1+*. These results indicate that in all true revertants of both strains tested there is only one copy of the *ade1* gene reverting to prototrophy and segregating into the viable spores, evidence that the monosomic chromosome in both strains is chromosome I.

DNA per cell

The amount of DNA per cell in the two strains monosomic for chromosome I and the diploid DK8 was determined by the diphenylamine method (15) to eliminate the possibility that the increase in rDNA in X1221a-7c 10/29 was due to the loss of any cellular DNA component that does not hybridize rRNA, thereby raising the percent DNA hybridized while not actually affecting the number of rRNA genes. If the increase in rDNA was real, we would expect the variations in total cellular DNA to be small because neither the absence of one copy of chromosome I nor the extra copies of rRNA genes should significantly contribute to the total amount of DNA per cell. The three strains tested gave approximately equal values (Table 3), which

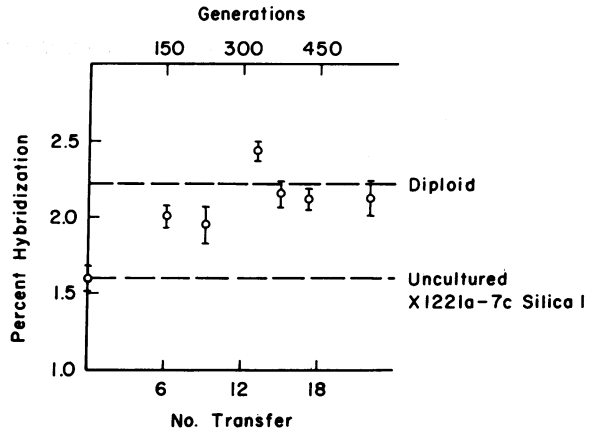

FIG. 1. Kinetics of the rDNA increase. A single colony of X1221a-7c silica 1 was transferred every 5 days on YEPD agar. The amount of rDNA was determined by 18S and 26S rRNA·DNA saturation hybridization for single colonies transferred a different number of times. Dashed lines represent values obtained for a colony of X1221a-7c silica 1 that was not transferred, and the diploid, DK8 silica 1. Each point represents the average of six determinations, each containing three filters per DNA preparation. Bars delineate the 95% confidence limits of the mean.

were all in close agreement with previously published results for the amount of DNA in diploid cells (17). It is not clear whether the slightly lower value obtained for X1221a-7c silica 1 was significant and caused by the missing copy of chromosome I. These results provide evidence that the actual number of rRNA genes has increased in X1221a-7c 10/29.

Kinetics of rDNA increase

Because the increases in rDNA levels were found in strains that had been serially cloned, we attempted to repeat the observation under more controlled conditions. A single colony of X1221a-7c silica 1 was serially transferred at 5-day intervals on a YEPD agar plate. Transfers were made for a period of 4 months until a series of plates had been obtained where cells had undergone from 25 to 550 generations. The rDNA level was measured by saturation rRNA·DNA hybridization in colonies that had been transferred a different number of times (Fig. 1). An increase in the amount of rDNA appeared after a maximum of six transfers (approximately 150 generations, estimating a single colony to be the product of 25 generations). The level of rDNA appears to have reached or slightly exceeded the diploid level by the thirteenth transfer. Eventually, the number of rRNA genes seems to stay constant at or near the level measured for the diploid DK8. All single colonies isolated were tested and shown to be monosomic as judged by 2:2, viable:inviable first division segregation. The rDNA level increased either continuously at a slow uniform rate or in a few large steps. Although the colonies picked at the sixth and eighth transfers appear to contain the same intermediate levels of rDNA, indicating the level of rDNA had increased half way to the diploid level and remained stable, the inaccuracies of the hybridization do not exclude the alternate possibility. Furthermore, we do not know if this increase occurred in all cells or just in the ones selected.

DISCUSSION

An apparent increase in the number of rRNA genes in a strain initially deficient for approximately 30% of these genes has been repeatedly observed in cells that have been repeatedly cloned. This phenomenon may be analogous in several respects to the

magnification of the rRNA genes in *Drosophila melanogaster bobbed* mutants. Unlike *Drosophila*, yeast strains monosomic for chromosome I that differ in their rRNA gene number show no obvious phenotypic differences. Preliminary evidence suggests that X1221a-7c 10/29, which shall be called the magnified monosome, and X1221a-7c silica 1, which shall be called the unmagnified monosome, look the same, grow at approximately the same rate, and contain the same amount of RNA/cell (Kaback and Katcoff, unpublished data). The magnified monosome and diploid strains of *S. cerevisiae* contain about 280 rRNA genes while the unmagnified monosome contains approximately 200. Thus, rDNA magnification in yeast involves approximately 80 genes. This deficiency is small when compared to the deficiencies found in *Drosophila*, which often involve greater than 50% of the total rDNA (7), and may be too small to cause an easily recognized phenotypic difference between the magnified and unmagnified monosomic strains. Alternatively, yeast may have a mechanism that compensates for the missing rRNA genes.

We consider the events in yeast similar to rDNA magnification and not rDNA compensation because the increased amount of rDNA in yeast appears stable although in a unicellular organism no real distinction between magnification and compensation is possible. In no case has the level of rDNA become reduced to the level in the unmagnified monosome. Furthermore, at least part of the rDNA accounting for magnification is transmitted through two meiotic cycles into viable haploids. We also have evidence that suggests that the magnified rDNA is chromosomally linked (Kaback and Halvorson, unpublished data).

It is not clear how the magnified monosomes arose in the population of unmagnified cells because it is not known whether the magnified monosome has a selective advantage over the unmagnified monosome. If there is a selective advantage, it must be subtle because both magnified and unmagnified monosomes grow at approximately the same rate. It is conceivable that a small proportion of magnified cells may be present in a population of unmagnified cells. The appearance of a magnified colony would then be the result of cloning one of these magnified cells. It is, therefore, necessary to determine the proportions of cells in the population that have magnified rDNA. Unfortunately, in the absence of an easily recognized phenotype for different levels of rDNA this becomes difficult.

Several of the mechanisms proposed to account for rDNA magnification in *Drosophila* may also be applicable to yeast, such as unequal crossingover (9, 18) or insertion of an rDNA-containing episome (19). Another mechanism that should be considered involves the development of the ability to replicate rDNA early in the cell cycle, before the rest of the chromosomal DNA. This would be in contrast to normal diploid yeast cells which replicate rDNA at the same time as the bulk of nuclear DNA (20). We consider this mechanism unlikely because all rDNA measurements were made using stationary phase cells which have presumably finished replicating their chromosomal DNA. At this stage any increase in the relative amount of rDNA per cell, which takes place early during the S period, would not be present, though we cannot exclude the possibility that an extra round of rDNA replication has occurred after the cells have stopped dividing.

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