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# Sedimentation Properties of Yeast Chromosomal DNA

**(eukaryote/high molecular weight DNA/unineme/spheroplast)**

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**ABSTRACT** **Sedimentation analysis of nuclear DNA released from spheroplasts of the yeast *Saccharomyces cerevisiae* indicates that it has a number average molecular weight of $6.2 \times 10^8$. The chromosomal DNA molecules range in size from as small as $5 \times 10^7$ daltons to as large as $1.4 \times 10^9$ daltons. Based on these values and estimates of the total DNA content of the yeast nucleus, it is proposed that each yeast chromosome contains a single DNA duplex.**

The average DNA content of eukaryotic chromosomes can be calculated if the DNA content of the nucleus and the number of the chromosomes are known. A human sperm contains $2.44 \times 10^{-12}$ g DNA (1) and 23 chromosomes, giving an average DNA content of about $6 \times 10^{10}$ daltons per chromosome. If there is a single DNA molecule in a eukaryotic chromosome (2), the molecule is very large compared to the DNA molecules that have been examined in most detail: those of bacteria, viruses, and cellular organelles. Microbial eukaryotes appear to be exceptions. Estimates of the amount of DNA per haploid nucleus of the yeast *Saccharomyces cerevisiae* range from $8.4 \times 10^9$ to $12.0 \times 10^9$ daltons (3) and, if there are 17 chromosomes, as seems likely (ref. 4 and D. C. Hawthorne, personal communication), the average yeast chromosome contains between $4.9 \times 10^8$ and $7.1 \times 10^8$ daltons of DNA. If the value is taken as $7.0 \times 10^8$ daltons, then this DNA mass is four times smaller than the *Escherichia coli* DNA chromosome and only six times larger than phage T4 DNA. It seemed possible, therefore, that physical techniques such as sedimentation velocity analysis might be applicable to the study of yeast chromosomal DNA. One of the primary difficulties in analysis of DNA molecules of this size is that they are readily broken by shear forces produced by pipetting and other methods of transfer. To avoid this problem, we have examined the sedimentation pattern of yeast nuclear and mitochondrial DNA released from spheroplasts that lyse after being layered on sucrose gradients containing salt and detergent. Proteins bound to yeast nuclear DNA are readily dissociated under these conditions (5). Some evidence for large yeast DNA molecules was obtained previously (6).

## MATERIALS AND METHODS

*Strain and Medium.* The strain of yeast used in these experiments was A364A D-5, provided by L. Hartwell. The strain is a diploid, auxotrophic for uracil (*ura1*) and adenine (*ad1*). The growth medium was Y-minimal, which contains (per liter) 6.7 g Difco yeast nitrogen base (with amino acids), 10 g succinic acid, 6 g NaOH, and is supplemented with 20 g glucose, 20 mg adenine, and 20 mg uracil per liter. The final pH was adjusted to 5.8. Cultures were grown aerobically by incubation in a rotary waterbath at 30°.

*Labeling.* RNA and DNA were labeled by addition to the medium of [6-$^3$H]uracil (New England Nuclear) to give 1 mCi/mg, or [2-$^{14}$C]uracil (Schwarz) to give 0.025 mCi/mg. Protein was labeled by addition of [*U*-$^3$H]tyrosine (New England Nuclear) to give 10 mg/liter and 1 mCi/mg. In experiments in which only the mitochondrial DNA was to be labeled, the procedure of Grossman *et al.* (7) was followed. We treated the cultures with cycloheximide (200 μg/ml) for 45 min before adding label and continuing incubation for 6 hr.

*Spheroplast Formation.* Cells for spheroplasting were harvested in exponential phase at a cell density of $2 \times 10^8$ cells/ml and washed two times with water. The method for spheroplast formation is that of Duell *et al.* (8), with minor modifications. The washed cells were suspended at $5 \times 10^8$ cells/ml in a solution containing 0.1 M sodium citrate buffer (pH 5.8), 0.9 M sorbitol, 0.01 M sodium-EDTA, 0.03 M mercaptoethylamine, and 2% (v/v) glusulase (Endo), and incubated at 37° for 30 min.

*Sedimentation Velocity Experiments.* A suspension of spheroplasts (100 μl) was layered directly onto a linear 15-30% sucrose gradient (4.7 ml). The sucrose solutions contained 1% sodium dodecyl sarcosinate (Sarcosyl), 0.01 M sodium-EDTA (pH 8), 0.01 M Tris·HCl (pH 8), and 1 M NaCl. For a sedimentation marker, T4 particles containing [$^{14}$C]DNA or λ particles containing [$^3$H]DNA were incubated with 5% Sarcosyl at 60° for 10 min. A large-bore pipette was used to transfer the released DNA ($2 \times 10^{-3}$ μg) to the gradient to avoid breakage by shear. T4 DNA molecules produced in this way are unbroken, as shown by their cosedimentation with nonradioactive T4 DNA characterized with an analytical ultracentrifuge as having a $s^0_{20,w} = 62$ (9). The sucrose gradients were incubated at room temperature for 15 min after addition of the spheroplasts and marker DNA. Lysis was complete in less than 5 min. RNase treatment was performed during spheroplast formation by addition of 100 μg/ml of pancreatic ribonuclease (Worthington). DNase treatment involved addition of 100 μg/ml of pancreatic deoxyribonuclease (Worthington) to the spheroplasting solution (without EDTA).

Gradients were run in a Spinco SW 50L rotor, which produces a centrifugal force of $8161 \times g$ at 10,000 rpm at the midpoint of the sample tube. Sedimentation runs were done at 5° at speeds between 8,000 and 25,000 rpm. After centrifugation, fractions were collected from the bottom of the tube, and the RNA was hydrolyzed in 1.0 ml 0.3 N NaOH (containing 1 mg/ml uracil) for 24 hr at 37°. The samples were chilled,

neutralized with HCl, and brought to 5% trichloroacetic acid. Precipitates were collected on glass-fiber filters (Whatman GF/C) and washed six times with 10 ml cold 5% trichloroacetic acid and three times with 10 ml cold 95% ethanol. The filters were dried and counted in a toluene-based scintillation fluid.

*Calculation of Molecular Weights.* Number average molecular weight was calculated as $M_n = 1/\Sigma\ (c_i/M_i)$, where $c_i$ = % cpm in a gradient fraction and $M_i$ = the molecular weight of DNA in that fraction. The relative molecular weight of material in a fraction was calculated by the equation of Burgi and Hershey (10), $S_1/S_2 = (M_1/M_2)^{0.35}$. For computation of $M_n$, it was necessary to exclude the "tail" of counts of yeast and phage DNA at the top of the gradient. This "tail" includes about 5% of the total yeast nuclear DNA. To make certain that this equation applies to DNA molecules sedimenting under our conditions, we determined the molecular weight of $T_4$ [$^{14}$C]DNA relative to that of λ [$^3$H]DNA by applying the equation to sucrose gradient sedimentation data. The results (Table 1) show good agreement with the values expected for intact virus DNA molecules.

*Equilibrium Density Centrifugation.* Fractions collected from a sedimentation velocity gradient were added to saturated CsCl solutions. T4 [$^{14}$C]DNA was added to each tube as a density marker. The density of the solution was adjusted to 1.690 g/ml by addition of water. 4 ml of the final solution were added to a 5-ml cellulose nitrate tube, and mineral oil was added to the top. Centrifugation was performed in an SW 50L rotor at 30,000 rpm, 20°, for 48 hr. The fractions were collected from the bottom of the tube and processed as for sucrose gradients.

## RESULTS

The effect of rotor speed on the sedimentation pattern of yeast DNA in the sucrose gradients is illustrated in Fig. 1. At 25,000 rpm (Fig. 1*A*), the pattern is similar to that observed previously (6), showing a fairly sharp peak with a sedimentation coefficient 1.49 times that of the T4 marker DNA. Intact T2 DNA sediments with a value of 57 S in sucrose gradients (11) and, taking this as the value for our T4 DNA, the peak fraction of yeast DNA and Fig. 1*A* is 85 S. As the rotor speed is decreased, the yeast DNA exhibits a higher average S value and a broader distribution in the gradient. Fig. 1*B* shows the pattern obtained at 10,000 rpm. Between 8,000 and 12,000 rpm, the pattern and average S value is constant. The sedimentation rate of the T4 DNA is not detectably affected by the rotor speed in the range 10,000–25,000 rpm. An effect of rotor speed on the sedimentation properties of large DNA molecules has been noted for other systems (13–15). Since the pattern of yeast DNA does not change below 12,000 rpm, we presume that this pattern reflects DNA molecules of various molecular weights sedimenting with S values as described by the equation of Burgi and Hershey (10) (see also *Discussion*). For example, the material in gradient fraction 24 shown in Fig. 1*B* has a sedimentation rate of 134 S. If the molecular weight of T4 DNA is $1.2 \times 10^8$ (11, 12), the molecular weight of the DNA in this fraction is calculated to be $1.4 \times 10^9$.

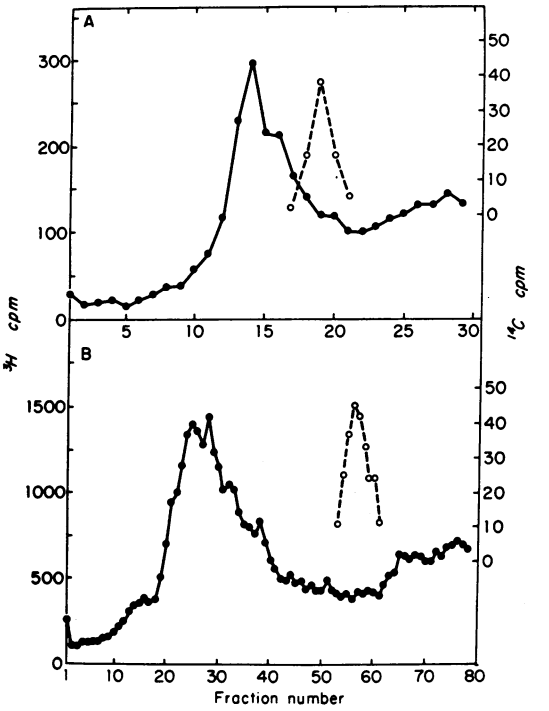


FIG. 1. Sedimentation pattern of yeast DNA at high and low rotor speeds. (●——●) yeast [$^3$H]DNA; (O- - -O) T4 [$^{14}$C]DNA. (*A*). Centrifugation at 25,000 rpm for 5 hr. (*B*) Centrifugation at 10,000 rpm for 26 hr.

TABLE 1. *Sedimentation of T4 DNA and λ DNA in sucrose gradients and estimation of relative molecular weights**

| Centrifugation (hr) | $d_{T4}$ | $d_\lambda$ | $d_{T4}/d_\lambda$ | $M_{T4}/M_\lambda$ | $M_{nT4}/M_{n\lambda}$ |
|---|---|---|---|---|---|
| (Expected) | — | — | 1.63 | 4.00 | 4.00 |
| 3 | 0.23 | 0.14 | 1.64 | 4.11 | 3.83 |
| 6 | 0.44 | 0.26 | 1.69 | 4.48 | 4.24 |
| 12 | 0.84 | 0.51 | 1.65 | 4.18 | 4.22 |

* T4 [$^{14}$C]DNA (1400 cpm) and λ [$^3$H]DNA (15,000 cpm) were cosedimented in 15–30% sucrose gradients at 25,000 rpm at 5°. The distance (*d*) that the peak of phage DNA has sedimented is given as a fraction of the total gradient. $M_{T4}/M_\lambda$ was calculated from $(d_1/d_2) = (S_1/S_2) = (M_1/M_2)^{0.35}$ (10). The relative number average molecular weights ($M_n$) were calculated as described in *Methods*, by the same equation. The *Expected* values were calculated with $1.2 \times 10^8$ and $3.0 \times 10^7$ for the molecular weights of T4 DNA (11, 12) and λ DNA (12), respectively.

The sedimentation of yeast DNA (Fig. 1*B*) does not appear to be influenced by DNA–DNA interactions or by interactions with other macromolecules. It is independent of DNA concentration in the range of 0.05–2 μg DNA/ml of lysate. Moreover, when $^{14}$C-labeled yeast DNA is sheared, mixed with $^3$H-labeled spheroplasts, and sedimented, the [$^{14}$C]DNA is present in the top quarter of the gradient. The control [$^4$H]-DNA and [$^{14}$C]DNA released from spheroplasts on the gradient gave the same sedimentation patterns as the yeast DNA in Fig. 1*B*. With the spheroplasting conditions used, degradation of the DNA by cellular enzymes or glusulase appears not to occur. This is shown by the fact that the sedimentation pattern is unchanged when the centrifugation is done immediately after spheroplast formation or after several

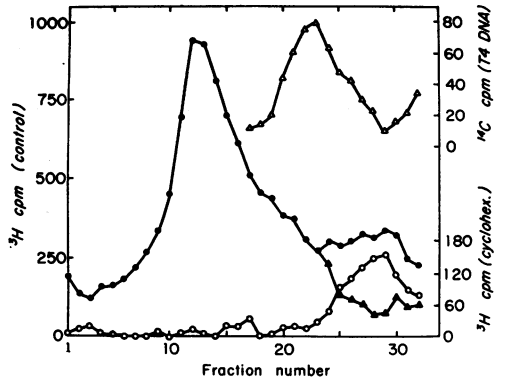


FIG. 2. Sedimentation pattern of yeast nuclear and mitochondrial DNA. (Δ——Δ) T4 [¹⁴C]DNA; (●——●) total yeast [³H]DNA control; (O——O) yeast mitochondrial DNA labeled with [³H]uracil during cycloheximide treatment; (▲——▲) total yeast [³H]DNA adjusted to show the pattern of nuclear DNA. The adjustment was made by taking the pattern of mitochondrial DNA in the upper 1/4 of the gradient, normalizing the counts to 65% of the total control DNA in that region, and subtracting these values from the total yeast DNA. 65% of the DNA in that region is mitochondrial DNA, as shown by CsCl banding experiments. Sedimentations were at 10,000 rpm for 26 hr.

hours at room temperature. Treatment with RNase during spheroplasting degrades over 95% of the RNA without affecting the DNA sedimentation pattern. Analysis of spheroplasts labeled with [³H]tyrosine and [¹⁴C]uracil shows that less than 0.034% of the total cell protein sediments in the region of the gradient with the main DNA peak (data not shown). As expected, most of the [³H]protein is found at the top of the gradient after centrifugation; however, some extends down into the region of fast-sedimenting DNA. This small amount of radioactivity does not appear to represent material associated with the DNA, since degradation of >98% of the DNA with DNase before centrifugation leaves the [³H]protein profile unaltered.

The yeast DNA distributed through the sucrose gradient after sedimentation includes both nuclear and mitochondrial DNA. The distribution of the two kinds of DNA was studied in two ways. One approach took advantage of the observation (7) that DNA labeled in cells pretreated and incubated with cycloheximide is, as shown by CsCl isopycnic banding, at least 90% mitochondrial DNA. We have confirmed this observation, and the sedimentation pattern of the labeled DNA released from the spheroplasts is shown in Fig. 2. As expected from measurements of the size of yeast mitochondrial DNA (6, 16), the radioactivity is found in the top portion of the gradient. The second approach to localization of mitochondrial and nuclear DNA in the sedimentation gradient was to band DNA fractions isopycnically in CsCl, where mitochondrial DNA (1.683 g/ml) is separated from nuclear DNA (1.699 g/ml). The results of such an experiment are shown in Fig. 3. These experiments show that mitochondrial DNA is confined to the upper 1/4 of the gradient, but a small fraction of 1.699 g/ml DNA, presumably of nuclear origin, extends into this region of the gradient. About 65% of the DNA in this part of the gradient is mitochondrial DNA.

The distribution of nuclear DNA and its number average molecular weight ($M_n$) can be estimated from the data in Figs. 2 and 3. If we assume that the sedimentation pattern of mitochondrial DNA is not altered in cells treated with cycloheximide, the sedimentation profile of total yeast DNA can be adjusted to obtain a profile of exclusively nuclear (1.699 g/ml) DNA (Fig. 2). The $M_n$ of this yeast nuclear DNA was calculated (see *Methods*) by comparison with T4 DNA, excluding from the calculation a "tail" of slow-sedimenting material for both DNAs. The $M_n$ value expected if there is one DNA duplex per chromosome in yeast should be between $4.9 \times 10^8$ and $7.1 \times 10^8$. The $M_n$ calculated from the data is $6.2 \times 10^8$.

## DISCUSSION

Although the possibility cannot be completely eliminated that yeast DNA is complexed with non-DNA material and consequently condensed to give high sedimentation values, we believe that the sedimentation profile represents free, extended DNA molecules for the following reasons. The fast-sedimenting DNA is extremely sensitive to breakage by shear forces, as expected for large, free DNA molecules. We are unable to recover the material intact from centrifugation tubes. Protein–DNA complexes formed by electrostatic interactions should be dissociated by the high salt concentration (1 M NaCl) and detergent (1% Sarkosyl) in the gradients. Van der Vliet *et al.* (5) found that at least 90% of the basic proteins of yeast deoxyribonucleoprotein was dissociated from the DNA by 0.8 M NaCl, and that the sedimentation coefficient of the DNA treated in this way decreases to the value found for completely deproteinized DNA. We do not observe a peak of protein sedimenting with the nuclear DNA. The sedimentation pattern of the small amount of protein sedimenting at a high rate is unaffected by degradation of the DNA with

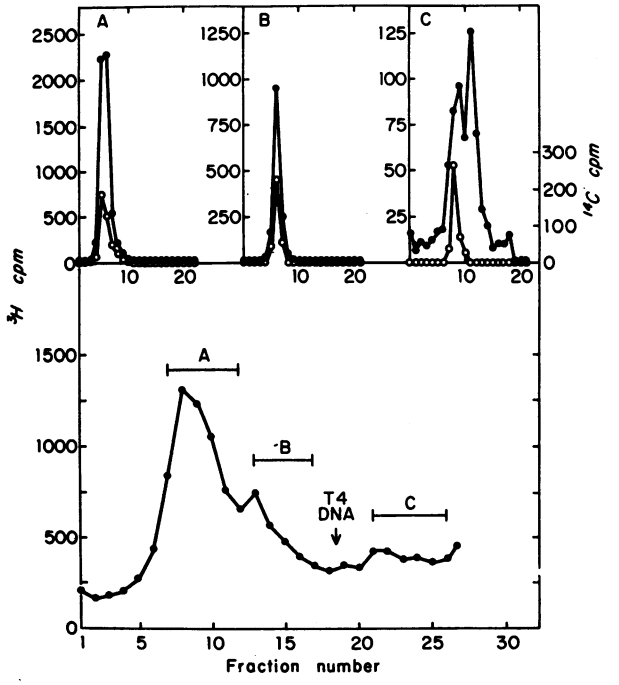


FIG. 3. Isopycnic analysis of DNA from a sedimentation velocity gradient. *Bottom panel:* Sedimentation pattern of total yeast [³H]DNA. Centrifugation at 10,000 rpm for 29 hr. Fractions were pooled as indicated for analysis by isopycnic banding in CsCl. *Top panels:* Isopycnic banding of pooled fractions *A*, *B*, and *C* in CsCl. (●——●) yeast [³H]DNA; (O——O) T4 [¹⁴C]-DNA (1.700 g/ml) marker.

DNase. RNA–DNA interactions do not appear to influence the DNA sedimentation, since >95% degradation of RNA by RNase has no effect. DNA–DNA aggregation does not appear to be a problem, since the sedimentation pattern is the same over a 40-fold concentration range and low molecular weight DNA produced by shear does not cosediment with the high molecular weight DNA.

The empirical equation of Burgi and Hershey (10) was used for conversion of S values of yeast chromosomal DNA molecules to molecular weights. The validity of the equation for phage DNA molecules sedimenting under our conditions was shown by experiments summarized in Table 1. T4 DNA and λ DNA molecules sediment at constant velocity, and the ratio of S values is that expected of their molecular weights and the Burgi-Hershey equation. We have assumed that the nuclear DNA molecules are linear and that the Burgi-Hershey equation is valid for large DNA molecules (up to $1.4 \times 10^9$ daltons) sedimenting at the lower rotor speeds. Support for the latter assumption has been provided by Kavenoff (13) in recent experiments in which the equation was applied to the sedimentation coefficient of *Bacillus subtilis* DNA obtained by extrapolation to zero rotor speed. The value obtained, 195 S, gives a molecular weight ($2.5 \times 10^9$) in good agreement with values obtained by more direct methods. The assumption is also supported by the experiments of Lehmann and Ormerod (14), in which the sedimentation properties of high molecular weight ($>2 \times 10^8$) mammalian DNA was examined. Their DNA preparation was treated with x-rays and consisted, presumably, of a random distribution of DNA molecular weights. The sedimentation pattern obtained at lower rotor speeds (10,000 rpm) was that expected for such a random collection of molecular weights. However, at higher rotor speeds (40,000 rpm) the DNA showed a sharp sedimentation pattern and lower average S value.

The number average molecular weight, $M_n$, determined for yeast nuclear DNA ($6.2 \times 10^8$) is about that expected if there is one DNA molecule per chromosome. The amount of DNA in the haploid nucleus of *S. cerevisiae* is variously reported to be from 8.4 to $12.0 \times 10^9$ daltons (3). If these values are divided by 17, the number of chromosomes reported for *S. cerevisiae* (ref. 4 and D. C. Hawthorne, personal communication), the average DNA content per chromosome is from 4.9 to $7.1 \times 10^8$ daltons.

We are assuming that the sedimentation profile of nuclear DNA represents a composite of unique DNA molecules partially separated in the centrifugal field. The sedimentation velocities of nuclear DNA in various fractions of Fig. 2 range down to at least 44 S (fraction 25) and up to at least 127 S (fraction 12), corresponding to molecular weights of 0.58–12.0 $\times 10^8$. If there is one DNA duplex per chromosome, an analysis of available genetic data indicates that such a broad range of molecular weights is expected. There are 132 known genetic markers distributed among the 17 chromosomes of *S. cerevisiae* (ref. 4 and D. C. Hawthorne, personal communication). The distribution of markers on the 17 chromosomes is significantly different from a normal distribution, and is that expected of a heterogeneous array of chromosome lengths, on the assumption that the number of genetic markers per chromosome is proportional to the physical size of each chromosome. The number of markers ranges from two on chromosome I to nineteen on chromosome IV, and the DNA molecular weights should exhibit a similar range of values. Given the range of values reported for the DNA content of the haploid nucleus, chromosome IV should be 19/132 (0.14) of this amount, or between 1.2 and $1.7 \times 10^9$ daltons, and this is the value found for the fastest-sedimenting fraction of nuclear DNA. Identification of DNA corresponding to specific chromosomes should be possible if we compare the sedimentation pattern of DNA from disomic ($n + 1$) strains with that from haploids ($n$) or diploids ($2n$). Such experiments are in progress.

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