Proc. Nat. Acad. Sci. USA
Vol. 70, No. 11, pp. 3072–3076, November 1973

Size and Structure of Yeast Chromosomal DNA

(electron microscopy/mitochondrial DNA/linear DNA)

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Communicated by Herschel L. Roman, July 5, 1973

ABSTRACT **Electron microscopic analysis indicates that yeast nuclear DNA can be isolated as linear molecules ranging in size from 50 μm (1.2×10^8 daltons) to 355 μm (8.4×10^8 daltons). Analysis indicates the data is consistent with the hypothesis that each yeast chromosome contains a single, linear DNA duplex. Mitochondrial DNA molecules have a contour length of 21 ± 2 μm and are mostly linear.**

The yeast *Saccharomyces cerevisiae* is a eukaryote with unusually small chromosomes, an average chromosome containing 5 to 7 $\times 10^8$ daltons of DNA. This value is based on a DNA content of the haploid nucleus of 0.92×10^{10} daltons (1) and the number (17) of genetic linkage groups (ref. 2). Their small size should make these chromosomes especially amenable to analysis by molecular techniques.

Sedimentation velocity experiments have provided evidence that yeast chromosomal DNA has an average molecular mass of about 6.2×10^8 daltons, with individual molecules ranging in size from about 0.5 to 12 $\times 10^8$ daltons (3). On the basis of these data we proposed that each yeast chromosome contains a single DNA duplex. The molecular weight values were, however, obtained from sedimentation values by means of an empirical equation that has not been shown to be valid for molecules of mass greater than 10^8 daltons. Moreover, it was assumed that the DNA molecules had a linear topology. We have now obtained a more direct estimate of the size of yeast chromosomal DNA molecules by electron microscopy. The data provide direct evidence for a linear topology and further demonstrate that yeast chromosomes are made up of single DNA duplexes. The size and topology of mitochondrial DNA molecules also were examined.

MATERIALS AND METHODS

Strains. The strain of yeast used in these experiments was A364A D-5, provided by L. H. Hartwell. The genotype of the strain has been described (3). A strain that lacks mitochondrial DNA was prepared from A364A D-5 by Dr. C. S. Newlon, using the technique of Goldring *et al.* (4).

Medium, Labeling, and Spheroplast Formation. These techniques have been described previously (3). RNA and DNA were labeled by incubating the cells with radioactive uracil (10 μg/ml).

Sedimentation Velocity Experiments. A suspension of spheroplasts (100 μl) was layered directly onto a linear 15–30% sucrose gradient (5 or 11.5 ml) containing 0.01 M sodium ethylenediaminetetraacetate (EDTA) at pH 8, 0.01 M Tris·HCl (pH 8), and 1 M NaCl. Spheroplasts were lysed on the gradient by addition of 50 μl of 5% sodium dodecyl sarcosinate (Sarkosyl).

After centrifugation, gradients were collected from the bottom of the tube through a large-bore (13G) needle (inside diameter, 1.9 mm), which had been boiled in 0.1 M EDTA. The flow rate was constant at less than 0.5 ml/min. Samples for measuring the amount of labeled DNA in each gradient fraction were incubated in 0.3 N NaOH for 24 hr at 37° to hydrolyze RNA (3).

Equilibrium Density Centrifugation. Fractions from sedimentation velocity gradients were adjusted to 1.690 g/ml with CsCl and 5 ml was transferred to a centrifuge tube. Centrifugation was in a Spinco type 40 rotor at 33,000 rpm and 20° for 48 hr.

Electron Microscopy. The procedure for Kleinschmidt spreading of DNA followed the aqueous method of Davis *et al.* (5) with precautions to reduce shear—transferring DNA by large-bore pipettes (1.6-mm internal diameter) at controlled slow rates of flow. The monolayer containing the DNA was picked up by touching it with Formvar-coated single-hole (0.8-mm diameter) grids, which were then dehydrated in ethanol, air dried, and rotary shadowed with platinum–palladium from an angle of 10°. Electron micrographs were obtained in a Philips EM 300 electron microscope and printed at a final magnification of 10,800. Contour length measurements were made with a Keuffel and Esser map measurer and converted to μm by calibration with phage DNA spread under identical conditions.

Bacteriophage DNA Standards and Calibration of Contour Lengths. DNA from bacteriophages T4, lambda, and lambda b2b5 was sedimented and prepared for electron microscopy under the same conditions as for the yeast DNA. Lambda wild-type·lambda b2b5 heteroduplexes were prepared by the method of Davis *et al.* (5).

Although the approximate contour length of a DNA molecule can be estimated with a diffraction grid, the dependence of contour length on the ionic strength of the spreading solution and other factors (5) necessitated calibration with DNA standards of known length. Bacteriophage T4 DNA was chosen as a standard because it has a well-characterized contour length and molecular weight, 52 μm and 1.2×10^8 respectively (6), and has a percent G + C, 34% (8), close to that of yeast nuclear DNA, 39% (9). The average contour length of 26 molecules of T4 DNA was 51.7 ± 2 cm at the magnification employed for the yeast nuclear DNA.

A second calibration was done to insure that the T4 DNA was unsheared. Lambda b2b5 (a phage carrying a deletion and a deletion–partial substitution) DNA was sedimented

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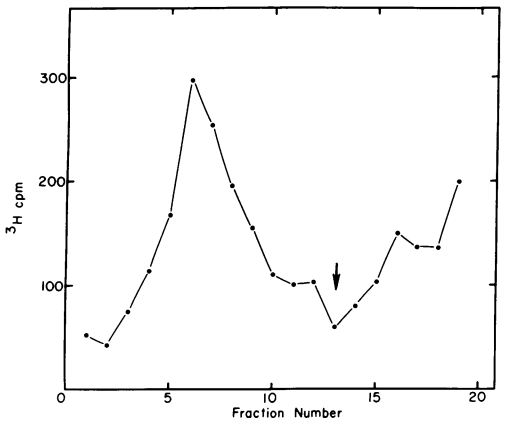

FIG. 1. Sedimentation pattern of total [^{3}H]DNA from yeast strain A364A D-5. Centrifugation at 9500 rpm for 26 hr in a Spinco SW50L rotor at 5°. *Arrow* indicates the peak of sedimentation of T4 DNA in a parallel gradient.

and spread for electron microscopy. The average contour length of 31 molecules was 14.5 ± 0.3 cm. The ratio of contour lengths for T4 DNA and lambda b2b5 DNA was 3.6. The expected ratio (6, 10) for intact DNAs is 3.66.

The lambda b2b5 was shown to be intact by constructing lambda wild-type·lambda b2b5 heteroduplexes. The relative positions of the single-stranded regions in the heteroduplex were the same as those observed by Westmoreland *et al.* (10). Consequently, the lambda b2b5 DNA is intact. Since the ratio of contour lengths indicates that the T4 DNA must also be intact, the length in μm for yeast nuclear DNA was determined by multiplying the ratio of contour length measurements of yeast nuclear DNA and T4 DNA by 52 μm.

RESULTS

Measurements of Contour Lengths of Yeast DNA Molecules from DNA Fractionated in a Sucrose Sedimentation Velocity Gradient. The DNA from a ^{3}H-labeled culture of yeast cells was centrifuged and collected in 19 fractions for electron microscopy. Each fraction was sampled to determine the sedimentation profile of the DNA (Fig. 1). The sedimentation pattern of the yeast DNA is very similar to that demonstrated previously (3, 11). DNA molecules from each gradient fraction (fractions 6–19) were prepared for electron microscopy, recorded on film, and measured. No DNA molecules were observed in fractions 16–19. Histograms of DNA contour lengths from fractions 6–15 (Fig. 2) suggest that shear may have reduced the expected lengths of the larger molecules.

Measurements of Contour Lengths of Unfractionated Yeast DNA. To reduce shear of the yeast DNA caused by collecting many fractions from a sucrose gradient, and to collect an unselected sample of yeast DNA molecules, we changed the fractionation procedure. We sedimented [^{3}H]DNA at high rotor speeds (35,000 rpm) on preparative sucrose gradients to separate the yeast nuclear DNA from more slowly sedimenting components. (3). The gradient was collected in two unequal fractions. Fraction I, the bottom fraction, contained 80% of the volume of the gradient and 92% of the total DNA. Fraction II, the top fraction, contained the remaining materials. The proportion of nuclear and mitochondrial DNA

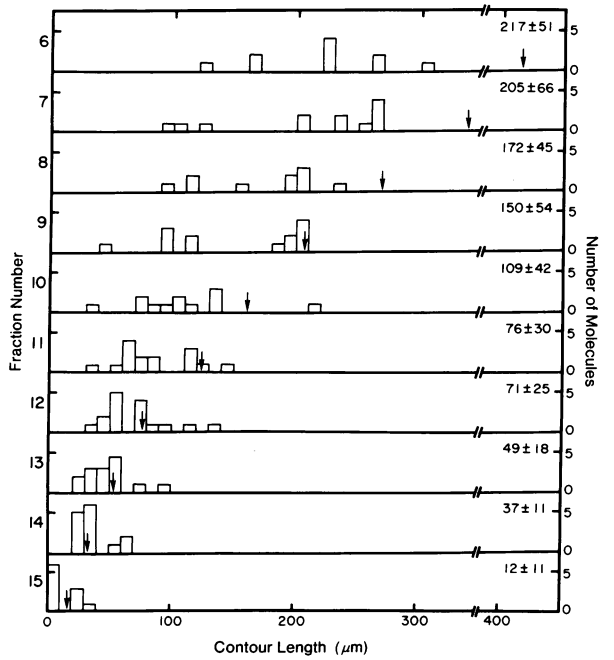

FIG. 2. Contour lengths of DNA molecules from fractions of the sucrose gradient shown in Fig. 1. The *arrow* in each individual histogram is the contour length expected on the basis of sedimentation data. Expected contour lengths are calculated by converting the expected molecular weights from each fraction to length measurements with the calibration established by Lang (6). Molecular weights (M) for each fraction of the gradient were calculated by comparing the distance sedimented by T4 DNA (d_1) with the distance sedimented by the yeast DNA in each fraction (d_2) using the equation: $(d_1/d_2) = (M_1/M_2)^{0.38}$ (7). Average contour lengths in μm for each fraction are on the right.

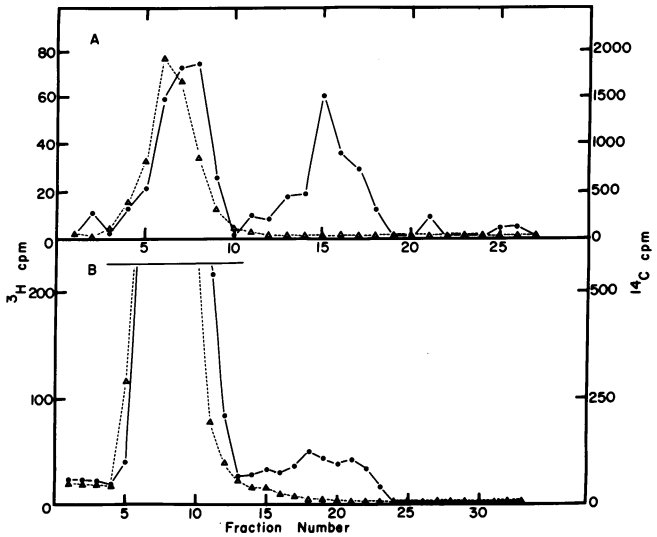

FIG. 3. Isopycnic analysis of yeast DNA. (A) Centrifugation to equilibrium in CsCl of yeast [^{3}H]DNA from the top 20% (fraction II) of a sedimentation velocity gradient. Yeast [^{14}C]-DNA from a mitochondrial DNA-less strain was added as a density marker. (B) Unfractionated yeast [^{3}H]DNA was centrifuged to equilibrium in CsCl with [^{14}C]DNA from a mitochondrial DNA-less strain. The peaks of nuclear DNA were normalized to the same height and truncated to allow visualization of the mitochondrial DNA. In (B), the total ^{3}H-cpm was 5966 and total ^{14}C-cpm was 12,765. (●), ^{3}H; (△), ^{14}C.

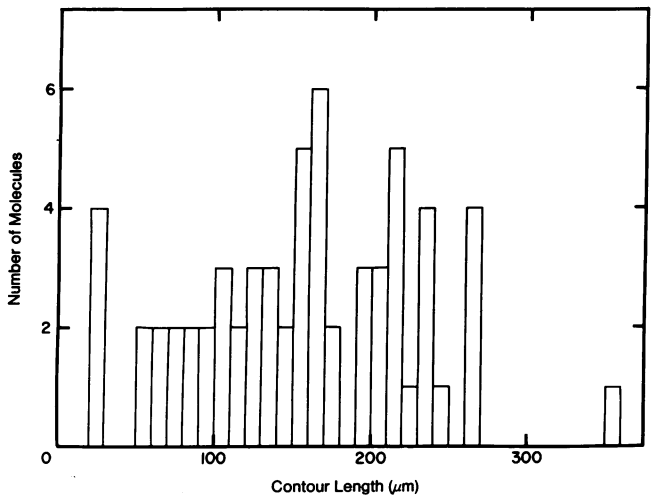

FIG. 4. Contour lengths of yeast DNA molecules isolated from a sedimentation velocity gradient (fraction I).

in these two gradient fractions was determined by equilibrium banding in CsCl, where nuclear DNA has a density of 1.699 g/ml and mitochondrial DNA has a density of 1.683 g/ml. Fraction I contained an undetectable amount of mitochondrial DNA (less than 2% of the total radioactivity); fraction II, as shown in Fig. 3, was 50% mitochondrial DNA. Therefore, 4% of the total DNA was accounted for as mitochondrial DNA in fraction II. Since this strain of yeast, under the growth conditions used in the experiment, contained 5% mitochondrial DNA (Fig. 3), most of the mitochondrial DNA was in fraction II. From these data, it can also be concluded that fraction I contained 96% of the nuclear DNA.

Fraction I was used for the contour length measurements shown in Fig. 4. Contour lengths could not be obtained from fraction II since the detergent in this fraction does not allow normal spreading of the DNA. For the measurements in Fig. 4, all DNA molecules which could be traced unambiguously were measured except the class of molecules with contour lengths of 2 μm. This class of molecules, originally described by Sinclair *et al.* (12), is probably extranuclear in origin (13). The contour lengths measured in fraction I range from 21 to 355 μm, with an average contour length of 165 μm. The average value probably represents a minimum because mechanical shear may have occurred during preparation for electron microscopy. A typical molecule, 165 μm long, is shown in Fig. 5. Most of the molecules (more than 95%) observed from fraction I were simple linear structures. Two types of exceptions were observed: Y-shaped molecules and molecules containing one or more internal "bubbles" or "eye" structures (Fig. 6), similar to those observed during T7 bacteriophage DNA replication (14). Procedures which are expected to enrich for replicating DNA molecules increase the proportion of these structures (Newlon, C. S., Petes, T. D., Hereford, L. M. & Fangman, W. L., in preparation). Therefore, yeast chromosomal DNA replication involves initiation at multiple sites within a molecule.

Contour Length Measurements of Yeast Mitochondrial DNA. To determine whether the 21-μm size class of DNA molecules in Fig. 4 represents nuclear or mitochondrial DNA, we sedimented in a single sucrose gradient [^{14}C]DNA from strain A364A D5 and [^{3}H]DNA from an equal quantity of cells of the mitochondrial DNA-less derivative of A364A D-5. Centrifugation was performed at a sufficiently high rotor speed and time to move the mitochondrial DNA away from the Sarkosyl layer (Fig. 7). In the part of the gradient containing most (94%) of the mitochondrial DNA, fractions 25–53 of Fig. 7, there is more than twice as much mitochondrial [^{14}C]DNA as non-mitochondrial [^{14}C]DNA. Twenty-one of 26 molecules measured from this fraction have a contour length of 21 ± 2 μm (Fig. 8). One of these 21-μm molecules had an open-circle configuration. 2-μm Circles and two-μm linear molecules were also present in this fraction. (There were about seven 2-μm molecules for each 21-μm molecule.) Since these molecules can account for the nonmitochondrial DNA that cosediments with mitochondrial DNA (Fig. 7), the 21-μm molecules observed in Fraction I (Fig. 4) must be predominantly mitochondrial in origin.

DISCUSSION

When individual fractions from a sucrose gradient are examined with the electron microscope, it is observed that the average contour length of the DNA molecules increases as fractions containing faster-sedimenting molecules are examined. The size of linear molecules observed in these fractions varied from 20 to 305 μm, corresponding to molecular masses of about 5 to 72 × 10^7 daltons. The DNA observed in these fractions, although very large, was smaller

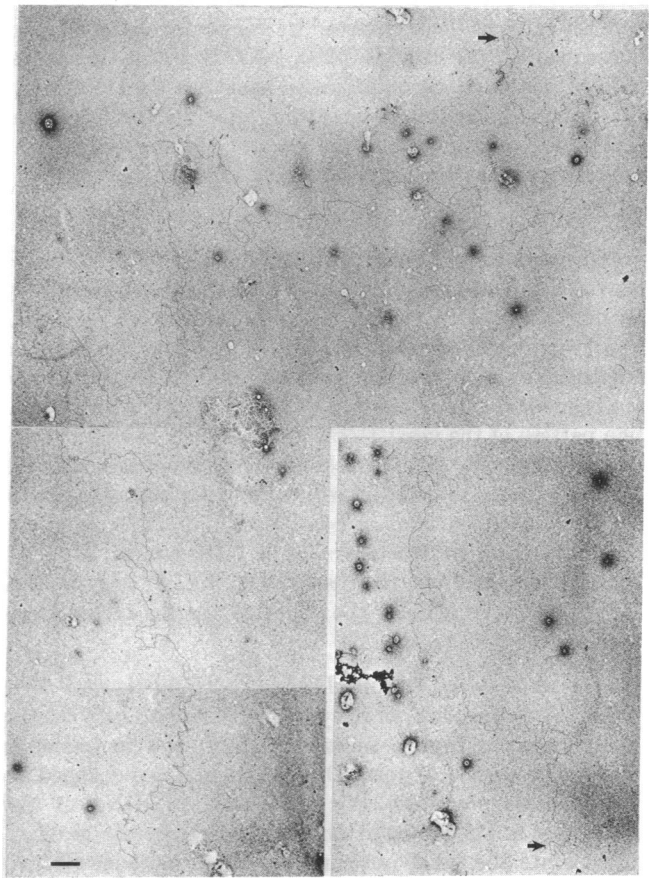

FIG. 5. Electron micrograph of a yeast nuclear DNA molecule 165 μm long. The figure was obtained from four overlapping micrographs containing the whole molecule. The *arrows* indicate regions of overlap of the molecule in the two panels. (*Bar* represents 1 μm.)

than expected from calculations based on empirical relationships between molecular weight and sedimentation coefficients (Fig. 2). This deviation became greater as faster-sedimenting fractions were examined. One obvious possibility is that shear of the largest DNA occurs during recovery of the gradient fractions or in preparation of the DNA for electron microscopy.

To minimize shear during recovery of the DNA and to obtain an unselected sample of DNA molecules in the yeast cells, we collected a single fraction which contained 96% of the nuclear DNA from a sucrose gradient. Conclusions concerning the size range and average size of yeast DNA molecules are based on data from this experiment (Fig. 4).

Since the 21-μm class of molecules is mitochondrial DNA, the size of nuclear DNA must range from 50 to 355 μm. Comparing the distribution of contour lengths in Fig. 4 with the average amount of DNA per yeast chromosome, we should be able to calculate the maximum number of DNA molecules per chromosome. The amount of DNA per chromosome can be determined by dividing the amount of DNA in a haploid yeast nucleus by the number of chromosomes. Since *S. cerevisiae* has a small genome and little redundant DNA (1, 15), the rate of renaturation has been used to estimate the genome size (15). The number of centromere-linked genetic linkage groups described in *S. cerevisiae* is 17 (2), which represents a minimum estimate of the number of chromosomes in a haploid set. An average chromosome, therefore, contains no more than 5.4×10^8 daltons of DNA. This molecular mass, when converted into a contour length from values for T4 DNA established by Lang [52 μm = 1.2×10^8 daltons (6)], yields an average total contour length of 230 μm of DNA per yeast chromosome. If there were two DNA molecules per chromosome, the expected average contour length would be 115 μm, whether the two DNA molecules of a chromosome were of the same size or of different sizes (if, for example, the chromosomal DNA were discontinuous at the centromere).

Because statistical tests† demonstrate that the mean of the contour length distribution (165 μm) of an unselected fraction of yeast nuclear DNA molecules exceeds the predicted mean for a model in which each chromosome contains two DNA molecules, at least some yeast chromosomes contain a single DNA duplex. Consequently, many of the DNA molecules observed in these studies must individually represent the entire DNA complement of a yeast chromosome.

Several generalizations can be made from these data. (*1*) The intact chromosomal DNA molecules isolated from yeast by these methods have a linear topology. It is possible, however, that large nuclear DNA rings exist within the cell but are broken during isolation. (*2*) The chromosomal DNA does not contain large single-stranded gaps. Our experiments with lambda·lambda deletion heteroduplexes indicate that gaps of 2 μm or more would have been detected as single-stranded "bushes". (*3*) At least some yeast chromosomes contain a single DNA molecule and are, therefore, unineme. Since the average contour length observed by microscopy is likely to have been reduced by shear, it is probable that every chromosome contains a single DNA helix. Moreover, because genetic mapping studies indicate that few of the yeast genetic linkage groups have a terminal centromere (2) the DNA helix must be continuous through the centromere in some, if not all, of the chromosomes. Similar conclusions have been reached by Kavenoff and Zimm (17) for *Drosophila* chromosomal DNA.

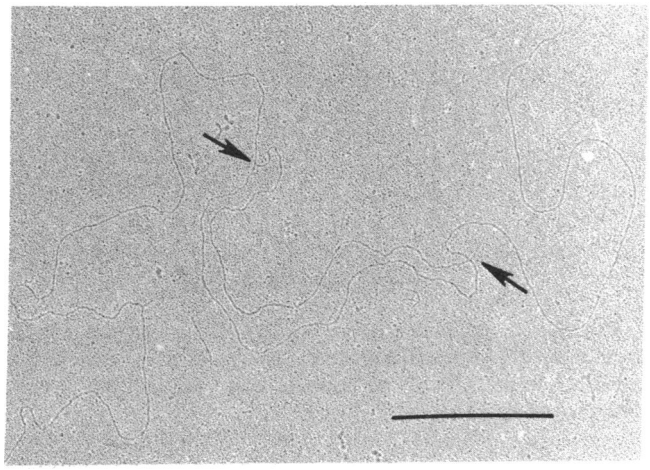

FIG. 6. Electron micrograph of a portion of a yeast nuclear DNA molecule containing a presumptive replication intermediate. The ends of the replication structure are indicated by *arrows*. The length of each arm of the "bubble" is 3 μm. The length of the whole DNA molecule is 75 μm. (*Bar* represents 1 μm.)

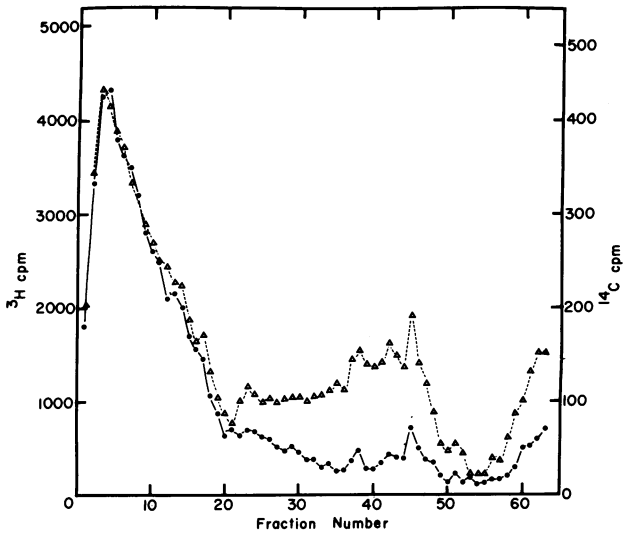

FIG. 7. Sedimentation pattern of total yeast [^{14}C]DNA from A364A D-5 and [^{3}H]DNA from the mitochondrial DNA-less strain of A364A D-5. Centrifugation was in a SW41 rotor, 22,000 rpm for 19 hr. (△), total yeast [^{14}C]DNA; (●) [^{3}H]DNA from strain lacking mitochondrial DNA.

† Two statistical tests were performed on the data. A *t*-test on the distribution of nuclear DNA shown in Fig. 4, testing the hypothesis that the true mean was 115 μm, gave a *t*-value of 6 with 57 degrees of freedom. The hypothesis can be rejected at a level of *P* much less than 0.5%. Although the *t*-test is strictly valid only for normal distributions, the test is relatively insensitive to departures from normality (16). A nonparametric test was also made on the median of the length distribution. If the hypothesis that the median of the distribution is 115 μm is tested, we find that 13 of 56 molecules are smaller than 115 μm. A chi-square analysis allows us to reject the hypothesis that 115 μm is the true median of the data at a level of *P* less than 0.5%. In addition, since the distribution of nuclear DNA shown in Fig. 4 is approximately symmetric (both the mean and the median are 165 μm), it is extremely unlikely that the mean of the distribution could be 115 μm.

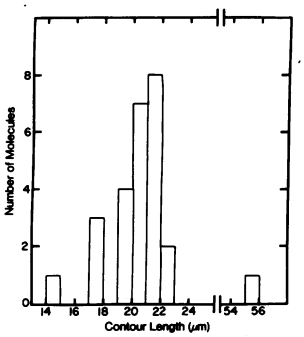

FIG. 8. Contour lengths of yeast DNA molecules isolated from a portion of a sedimentation velocity gradient enriched for mitochondrial DNA. The sample was obtained by pooling fractions 25 to 53 shown in Fig. 7

(4) In addition, centromeric regions in yeast chromosomal DNA do not remain unreplicated for a significant portion of the cell cycle after DNA synthesis. Chromatids of many higher organisms are held together after DNA replication until metaphase. One mechanism proposed for this behavior (18) is the delay of replication of a small portion of the chromosome near the centromere until metaphase. In the electron microscope, a DNA molecule from such a chromosome should have an X-configuration with two pairs of equally long arms. The observation that DNA from an asynchronous cell culture has no such molecules (0/250 molecules) indicates that the yeast chromosome probably does not retain this conformation for a significant part of the cell cycle.

Molecular size estimates by contour length measurements are in reasonably good agreement with measurements of chromosomal DNA sizes by sedimentation velocity experiments (3). The range of contour lengths (50–355 μm) corresponds to molecular masses of 1.2 to 8.4 $\times$ 10^8 daltons. The observation that no molecules in a sample of 58 had contour lengths between 25 and 50 μm indicates that molecules observed with these contour lengths in the first experiment (Fig. 2) were shear products or molecules present in the cell at low frequencies. Petes and Fangman (3) estimated the range of molecular masses from sedimentation data to be about 0.5 to 12–14 $\times$ 10^8 daltons. The fact that DNA molecules as large as 10^9 daltons (about 500 μm) were not found by electron microscopy may mean that the largest yeast chromosomal DNA molecules were sheared by mechanical forces during the preparation for electron microscopy, or that the empirical relationship used to convert sedimentation values to molecular weights in the sedimentation velocity experiments is not valid for molecules in this size range. We cannot distinguish between these two possibilities at present. Although only one molecule over 350 μm has been observed, five molecules longer than 300 μm have been measured in other experiments (Petes, unpublished observations).

Although there are conflicting reports concerning the size and topology of yeast mitochondrial DNA (12, 19–21) the most recent reports indicate that at least some yeast mitochondrial DNA molecules can be isolated as 25-μm closed twisted circles (22). Sedimentation experiments (3, 11) have also indicated that mitochondrial DNA, although smaller than nuclear DNA, is larger than early reports of contour lengths had indicated. The contour length of 21 μm found here (Fig. 8) agrees well with the estimate of the molecular mass of 46 $\times$ 10^6 daltons calculated from sedimentation data by Blamire *et al.* (11). The finding that most mitochondrial DNA is linear (only 1 out of 26 molecules was circular) may mean that circles are broken during isolation or that the circular form of mitochondrial DNA is present only transiently during the life cycle of a molecule.

This work was supported by grants from the National Science Foundation (GB-19792) and the National Institutes of Health (GM-18926, GM-18541). T.D.P. was supported by a National Institutes of Health Predoctoral Training Grant (GM-00182).

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