Proc. Natl. Acad. Sci. USA
Vol. 76, No. 12, pp. 6515–6519, December 1979
Genetics

Nucleosome organization of the yeast 2-μm DNA plasmid: A eukaryotic minichromosome

(chromosome structure/multiple copy plasmid/*Saccharomyces cerevisiae*)

RICHARD G. NELSON AND WALTON L. FANGMAN

Department of Genetics, University of Washington, Seattle, Washington 98195

Communicated by Herschel L. Roman, September 13, 1979

ABSTRACT **The eukaryotic microorganism *Saccharomyces cerevisiae* contains 50–100 copies per cell of a circular plasmid called 2-μm DNA. The intracellular structure of these molecules, which represent about 4% of the total DNA, was examined by digestion of total cellular chromatin with micrococcal nuclease (nucleate 3′-oligonucleotidohydrolase, EC 3.1.31.1). Nuclease-resistant DNA fragments were fractionated by gel electrophoresis and 2-μm DNA sequences were detected by hybridization. The 2-μm and chromosomal DNA digestion patterns were very similar indicating that both types of DNA are condensed into nucleosomes. An analysis of these digestion patterns showed that the kinetics of digestion of 2-μm chromatin and total chromatin are similar and that both have the same nucleosome repeat length of about 165 base pairs. Native 2-μm plasmids were examined by zone sedimentation in sucrose gradients containing 0.15 M NaCl and were found to have a sedimentation constant of 75 S, about 3 times the sedimentation constant of protein-free 2-μm DNA. This sedimentation property is what would be expected for a 2-μm DNA minichromosome. We conclude that within the cell 2-μm DNA molecules are organized in a chromatin structure very similar to that of the yeast chromosomes.**

Most strains of the yeast *Saccharomyces cerevisiae* contain 50–100 copies per cell of a circular 2-μm DNA plasmid (1). This 6-kilobase plasmid contains a 600-base pair nontandem inverted sequence repetition that divides the molecule approximately in half (2–7). The plasmid exists in two isomeric forms that differ in the orientation of unique-sequence DNA relative to the inverted repeat. These isomers are believed to result from intramolecular recombination within the inverted repeat region (8). It has been suggested that the plasmid copies are sequestered in nonmitochondrial cytoplasmic organelles (9–11). Although the plasmid has no known function, both strands are transcribed *in vivo* into poly(A)-containing RNA, which is found on polyribosomes (12).

The control of 2-μm plasmid replication appears to be identical to the control of chromosome replication. Like chromosomal DNA, and unlike mitochondrial DNA, 2-μm DNA replication is blocked in *a* mating type cells by the yeast mating pheromone α factor and is also prevented at the restrictive temperature in mutants of cell division cycle genes *cdc4*, *7*, or *28* (13). These treatments arrest yeast cells late in the G_1 phase of the cell cycle (14, 15). It has also been shown recently that, like chromosomal DNA, each 2-μm DNA molecule replicates once each S phase (16).

The similarities between chromosomal and 2-μm DNA replication raise the possibility that intracellular 2-μm DNA might be organized in a chromosome-like structure. In yeast, as in higher eukaryotes, chromosomal DNA is condensed with histone proteins into nucleosomal subunits (17–19). Exposure of nuclei or chromatin to micrococcal nuclease results in the preferential cleavage of chromosomal DNA in regions linking adjacent nucleosomes. This nuclease attack liberates nucleosome oligomers containing DNA fragments whose lengths are integral multiples of the yeast mononucleosome length of approximately 165 base pairs. If 2-μm DNA is also condensed by histone into nucleosomes and if such a minichromosome is accessible to the enzyme, it should be similarly digested. We have examined DNA fragments produced by micrococcal nuclease digestion of total yeast chromatin for sequences complementary to 2-μm DNA by using the DNA transfer-hybridization technique of Southern (20). Our results demonstrate that 2-μm DNA fragments are present in the same discrete multimeric size classes as those produced during the digestion of nuclear chromatin. In addition, intact 2-μm DNA released from yeast spheroplasts by osmotic lysis has the sedimentation constant expected for a compact nucleosomal structure. These results indicate that the 2-μm DNA plasmid is condensed into nucleosomal subunits and has a structure similar to the minichromosome of simian virus 40 (SV40) (e.g., ref. 21).

MATERIALS AND METHODS

Yeast and Bacterial Strains. Two strains of yeast containing 2-μm DNA (2-μm$^+$ strains), A364A and HQ/5C, and one strain lacking the plasmid (2-μm$^-$ strain), NCYC74-CB11 (9), were used. The 2-μm DNA of HQ/5C is about 5% smaller than that of A364A (9). *Escherichia coli* strain HB101 (82-6B) contains a recombinant plasmid in which a partial 2-μm dimer (9.8 kilobases) from strain A364A has been inserted at the *Eco*RI site of pMB9. Digestion of this plasmid with *Pst* I liberates unit-length linear 2-μm DNA. The strain was a gift of John Donelson.

Micrococcal Nuclease Digestion. Cells of A364A (2-μm$^+$ strain) in logarithmic or stationary phase cultures were harvested by centrifugation and converted to spheroplasts by removing the cell wall with glusulase (22). Spheroplasts were washed with digestion buffer (SPC) containing 1 M sorbitol, 20 mM 1,4-piperazinediethanesulfonic acid (Pipes) (pH 6.3), and 0.1 mM $CaCl_2$. Spheroplasts were resuspended in 0.25 ml of the same buffer per g of cells (initial wet weight) and added slowly along the side of a beaker to 40 vol of a stirred solution containing 9% (wt/wt) Ficoll 400, 20 mM Pipes (pH 6.3), 0.5 mM $CaCl_2$, and 1 mM phenylmethylsulfonyl fluoride ($PhMeSO_2F$). This suspension was centrifuged at 20,000 × *g* for 20 min and the supernatant was removed by aspiration. The pellet was washed in SPC containing 1 mM $PhMeSO_2F$, centrifuged at 12,000 × *g* for 10 min, and resuspended in 2 ml of the same buffer per g of cells (initial wet weight). This suspension was preincubated at 37°C for 2 min and micrococcal nuclease was added to 500 (Worthington) units per ml. At several times after

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

Abbreviations: $PhMeSO_2F$, phenylmethylsulfonyl fluoride; SV40, simian virus 40; Pipes, 1,4-piperazinediethanesulfonic acid; SPC, 1 M sorbitol/20 mM Pipes, pH 6.3/0.1 mM $CaCl_2$.

addition of enzyme, samples were placed on ice and digestion was stopped by adding EDTA to a final concentration of 20 mM.

Cells in a stationary-phase culture of NCYC74-CB11 (2-μm$^-$ strain) were converted to spheroplasts, washed twice in SPC containing 1 mM $PhMeSO_2F$ and resuspended in the same buffer at 3.5×10^7 cells per ml. Digestion was initiated by lysing samples of the spheroplast suspension in 20 vol of digestion buffer containing 5 mM potassium phosphate (pH 7.2), 0.1 mM $CaCl_2$, and 150 units of micrococcal nuclease per ml at 37°C, and immediately made 1 mM in $PhMeSO_2F$. Digestion was stopped as above.

Purification and Electrophoresis of Nuclease-Resistant DNA. Digested samples were made 1% in sodium dodecyl sulfate, vortex mixed, and centrifuged at $3000 \times g$ for 5 min. The supernatant was deproteinized once with (per ml of supernatant) 0.1 ml of 5 M $NaClO_4$/0.5 ml of a mixture of $CHCl_3$ and isoamyl alcohol, 24:1 (vol/vol)/0.5 ml of redistilled phenol saturated with 0.1 M Tris·HCl (pH 7.5), and then repeatedly extracted with 1 vol of $CHCl_3$/isoamyl alcohol until the interface was clear. The aqueous phase was precipitated with ethanol, resuspended in 0.15 M NaCl/15 mM Na citrate, and digested with 100 μg of RNase A per ml and 50 units of RNase T1 per ml at 37°C for 2 hr. Samples were deproteinized with $CHCl_3$/isoamyl alcohol, precipitated with ethanol, and resuspended in 15 mM NaCl/1.5 mM sodium citrate. Electrophoresis was carried out in 1.5-mm-thick 2% agarose slab gels in 40 mM Tris·HCl/5 mM NaOAc/1 mM EDTA at pH 7.8 for 5 hr at 5 V/cm. Gels were stained for 10 min in 4 μg of ethidium bromide per ml and photographed under ultraviolet illumination through combined red and yellow filters.

Preparation of Hybridization Probes. Purified 2-μm pMB 9 recombinant plasmid DNA from *E. coli* strain HB101 (82-6B) was the generous gift of Virginia Zakian. This DNA was cleaved with *Pst* I and the resulting 2-μm unit-length linear DNA fragment was isolated from a preparative 0.4% agarose gel by electrophoresing the fragment into hydroxyapatite (23). The DNA was eluted from hydroxyapatite at room temperature with 1.0 M sodium phosphate and desalted on a Bio-Gel P-100 column. The 2-μm DNA, recovered in the void volume, was precipitated with ethanol and dissolved in 10 mM Tris·HCl/10 mM NaCl at pH 7.5.

Chromosomal DNA was prepared from strain A364A by lysing spheroplasts from 10 g of cells in 50 ml of 3% sodium laurylsarcosinate (Sarkosyl)/0.2 M EDTA at pH 7.5 and 65°C for 10 min. Proteinase K (5 mg) was added to the lysate, which was then incubated at 55°C for 20 min. An additional 5 mg of proteinase K was added and incubation was continued for another 20 min. Portions (20 ml) of this lysate were then layered on 29-ml linear 5–20% sucrose gradients containing 1 M NaCl, 0.1 M EDTA, 0.1% Sarkosyl at pH 7.0 overlaying 10 ml of CsCl (1.70 g/ml)/0.1 M EDTA. The gradients were centrifuged in a Beckman SW 25.2 rotor at 20°C for 18 hr at 22,000 rpm. Fractions (5 ml) were collected from the tube bottom, and the viscous fractions containing the high molecular weight DNA were pooled and dialyzed against 0.15 M NaCl/15 mM Na citrate overnight. The DNA solution was made 1.70 g/ml in CsCl and centrifuged to isopynic equilibrium. Fractions containing the chromosomal DNA were pooled, dialyzed against 0.15 M NaCl/15 mM Na citrate, and treated with 50 units of RNase T1 per ml and 100 μg of RNase A per ml for 90 min at 37°C. The preparation was deproteinized with equal volumes of $CHCl_3$/isoamyl alcohol, precipitated with ethanol, and resuspended in 15 mM NaCl/1.5 mM Na citrate. Samples of both DNAs were labeled with α-^{32}P-labeled deoxyribonucleotide triphosphates by nick translation (24, 25). Specific activities ranged from 5×10^6 to 5×10^7 cpm/μg.

DNA Transfer and Hybridization. Nuclease-resistant DNA was fractionated by electrophoresis on agarose gels and transferred to Schleicher and Schuell BA 85 nitrocellulose filters with 0.9 M NaCl/90 mM Na citrate by the technique of Southern (20). Prior to hybridization, filters were soaked for 30 min at room temperature in 0.9 M NaCl/90 mM Na citrate containing Denhardt's solution (26). Hybridizations were carried out in a solution (75 μl/cm^2 filter area) containing 0.6 M NaCl, 0.18 M Na_2HPO_4 (pH 6.2), 6 mM EDTA, 1% Sarkosyl, Denhardt's solution, 50 μg of *E. coli* DNA carrier per ml and from 10^5 to 2×10^6 cpm of denatured (100°C for 5 min) probe DNA per ml. The nitrocellulose filters and hybridization solution were sealed in a plastic bag which was rocked in a 65°C oven for 18 hr. After hybridization, filters were washed twice for 60 min in 0.2 M NaCl/0.06 M Na_2HPO_4, pH 6.2/2 mM EDTA/1% Sarkosyl at 37°C and then four times in 10 mM Tris·HCl, pH 7.5, at room temperature. Filters were autoradiographed (Kodak XR-5 film) at room temperature or at −70°C when a DuPont Lightning-Plus intensifying screen was used.

DNA Fragment Size. DNA fragments from micrococcal nuclease digests were visualized by staining gels with ethidium bromide or by autoradiography of nitrocellulose filters. The fragments were sized by determining their mobilities relative to *Sau* 3a restriction fragments of 2-μm DNA (strain HQ/5C). These restriction fragments were electrophoresed in the same gel and transferred to the same nitrocellulose filter as the micrococcal nuclease digest. The sizes of the *Sau* 3a restriction fragments were determined by their mobilities relative to a *Hae* III digest of ϕX174 replicative form DNA on 4% acrylamide gels. Measurements of migration distance were made from Joyce–Loebl densitometer tracings of photographic negatives or autoradiograms, and calibration curves were constructed by linear regression. Calibrations of the molecular weights of two of the *Sau* 3a fragments relative to ϕX174 fragments varied significantly between agarose and acrylamide gels and these fragments were not used in constructing calibration curves.

Sedimentation of 2-μm DNA. A 300-ml culture of strain HQ/5C was labeled for two generations with 8 μCi of $^{32}PO_4$ per ml in low-phosphate medium (27). Cells were harvested at a density of 3×10^7 cells per ml and spheroplasts were prepared (22). The spheroplasts were divided into two equal portions and washed twice with 1 M sorbitol by centrifugation and resuspension. The spheroplasts were resuspended in about 0.1 ml of sorbitol and lysed by adding the suspension to 1.0 ml of lysis buffer containing 5 mM potassium phosphate (pH 6.5), 1 mM EDTA, 1 mM $PhMeSO_4F$, 25 mM 2-mercaptoethanol, with or without 0.2% (wt/vol) Nonidet P-40 (28). The lysates were placed on ice for 1 hr, an additional 1.0 ml of lysis buffer was added, and the lysates were centrifuged at $8000 \times g$ for 10 min. Samples (200 μl) were layered on 5-ml linear 5–20% sucrose gradients containing 150 mM NaCl, 1 mM EDTA, and 10 mM Pipes (pH 7.0). Centrifugation was at 4°C in a Beckman SW50.1 rotor for 115 min at 40,000 rpm. Identical results were obtained whether or not Nonidet P-40 was included in the lysis buffer.

The position of 2-μm [^{32}P]DNA in the gradients was determined by gel electrophoretic analysis of each gradient fraction and autoradiography of the dried gel. Ten microliters of 5% Sarkosyl/50% sucrose/0.02% bromphenol blue was added to 40 μl of each gradient fraction and these samples were loaded onto a horizontal 0.4% agarose slab gel. The gel and electrophoresis buffer contained 89 mM Tris·HCl, 89 mM boric acid, 2.5 mM EDTA, and 0.5 μg of ethidium bromide per ml at pH 8.0; electrophoresis was carried out at 1 volt/cm for 14 hr. The

gel was photographed and dried onto filter paper. The dried gel was treated with 0.5 M NaOH for 12 hr at room temperature to hydrolyze [^{32}P]RNA remaining in the gel, soaked for several hours with 0.5 M Tris·HCl (pH 7.0), rinsed with distilled water, and dried again. Dried gels were autoradiographed with Kodak XR-5 film at room temperature. Sedimentation constants were estimated by reference to λ DNA (34.4 S) (29) in parallel sucrose gradients.

RESULTS

Nuclease Digestion Patterns. A nucleosomal organization of 2-μm DNA within yeast cells was demonstrated by micrococcal nuclease digestion. Cell walls were removed from yeast cells containing 2-μm DNA (2-μm$^+$ strain) and the resulting spheroplasts were treated with $CaCl_2$ in the presence of Ficoll. Permeable spheroplasts prepared in this way were incubated with micrococcal nuclease for various times, and protected DNA was isolated and fractionated on agarose gels. The 2-μm DNA was detected among these DNA fragments after transferring them to nitrocellulose filters (20) and hybridizing with 2-μm [^{32}P]DNA. The 2-μm [^{32}P]DNA was also hybridized to nuclease-resistant DNA isolated from digests of the 2-μm$^-$ strain in order to detect any possible cross-hybridization of the 2-μm [^{32}P]DNA probe to chromosomal DNA.

Fig. 1*A* shows a photograph of an ethidium bromide-stained gel containing DNA isolated after digestion by micrococcal nuclease. Lanes 1, 2, and 3 contain DNA from the 2-μm$^-$ strain, and lanes 1′, 2′, and 3′ contain DNA from the 2-μm$^+$ strain. The pattern of digestion of total cellular DNA is similar for both strains. Because 2-μm DNA constitutes only about 4% of the total cellular DNA, it would not contribute significantly to the observed patterns. Fig. 1*B* shows the autoradiogram obtained when DNA from such a gel was transferred to a nitrocellulose sheet and hybridized to 2-μm [^{32}P]DNA. The ^{32}P probe hybridized to the full series of DNA fragments from the 2-μm$^+$ strain, but there was no hybridization to the fragments from the 2-μm$^-$ strain. It is clear, therefore, that the hybridization observed with the 2-μm$^+$ strain is specific for 2-μm DNA and that at least a portion of a cell's 2-μm DNA plasmids are organized into structures that protect discrete size classes of 2-μm DNA from micrococcal nuclease digestion. Such protection is characteristic of the condensation of discrete lengths of DNA into nucleosomes and, in fact, the 2-μm DNA digestion pattern is virtually identical to the pattern obtained from chromosomal DNA (Fig. 1 *A* and *C*, lanes 1′, 2′, and 3′), which is known to be condensed with histone into nucleosomal subunits. We conclude that intracellular 2-μm DNA is also organized into nucleosomes.

The 2-μm DNA Nucleosome. A comparison of the sizes of nuclease-resistant 2-μm and chromosomal DNA fragments was made by scanning autoradiograms with a densitometer and determining migration distances relative to standard restriction fragments. A comparison of these densitometric tracings (Fig. 2), as well as tracings of stained gel photographs (not shown), indicates that at each digestion time the size distribution of fragments from 2-μm DNA and chromosomal DNA are very similar. These results suggest that 2-μm and nuclear chromatins are digested by micrococcal nuclease at about the same rate. We cannot eliminate the possibility that a fraction of 2-μm DNA is not organized into nucleosomes and is preferentially digested early during the nuclease treatment. It is clear, however, that some 2-μm DNA exists in regularly spaced nucleosomal arrays of 10 or more units in length (Fig. 2*A*). The 2-μm DNA hybridization pattern (Figs. 1*B* and 2*A*) also reveals that intact supercoiled (form I) and relaxed (form II) molecules persist throughout the digestion period. This nuclease-resistant fraction of 2-μmDNA could represent a subset of intracellular molecules that are inaccessible to the nuclease. It is more likely, however, that these intact DNAs originate from a small fraction of the spheroplasts that remain impermeable to nuclease during digestion but are lysed during the DNA isolation procedure.

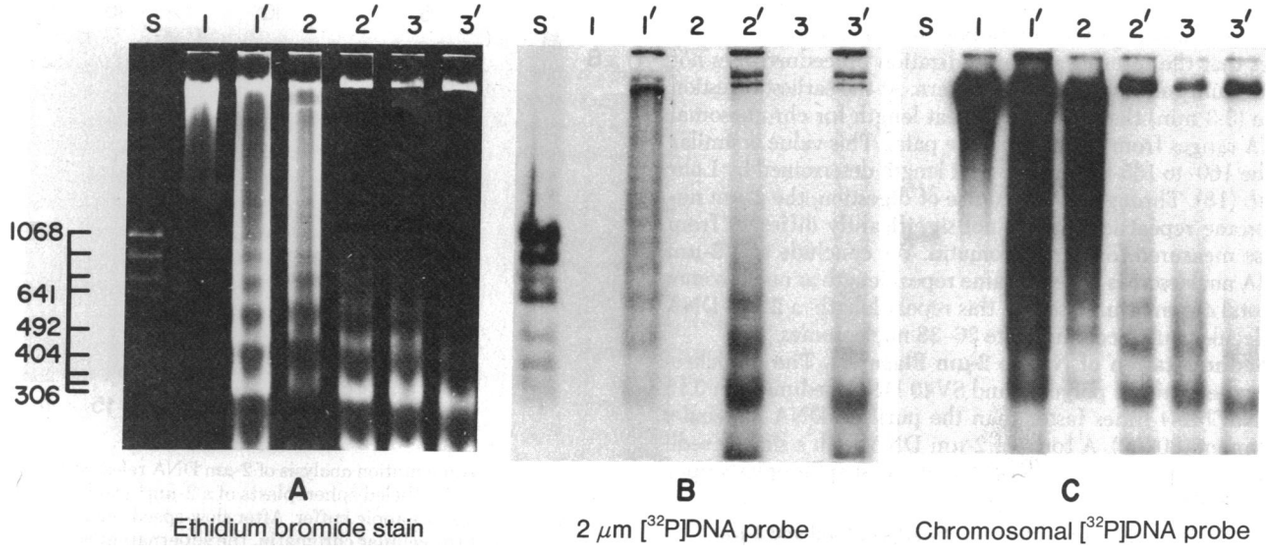

FIG. 1. Electrophoretic analysis of chromosomal and 2-μm DNAs after micrococcal nuclease digestion. (*A*) Photograph of an ethidium bromide-stained gel. (*B*) An autoradiogram of DNA transferred from a gel to nitrocellulose and hybridized with 2-μm [^{32}P]DNA. (*C*) An autoradiogram of DNA transferred from the gel shown in *A* to nitrocellulose and hybridized with chromosomal [^{32}P]DNA. In each panel, lanes 1, 2, and 3 contain DNA from a 2-μm$^-$ strain and lanes 1′, 2′, and 3′ contain DNA from a 2-μm$^+$ strain. In all cases the mononucleosome DNA fragments have been run off the gel. The times of digestion with micrococcal nuclease were 4 min (lane 1), 3.3 min (lane 1′), 8 min (lane 2), 10 min (lane 2′), 16 min (lane 3), and 30 min (lane 3′). Lane S in each panel contains *Sau* 3a-digested HQ/5C 2-μm DNA. The fragments indicated by the scale on the left were calibrated by comparison with *Hae* III-digested φX174 DNA and have the following sizes (base pairs): 1068, (923), (841), 641, 492, 404, 356, 320, 306, 231. The mobilities of the fragments in parentheses varied between acrylamide and agarose and they were not used in constructing calibration curves. The chromosomal [^{32}P]DNA probe hybridizes with the 2-μm DNA restriction fragments in lane S of *C* because the chromosomal DNA preparation was slightly contaminated with 2-μm DNA prior to nick translation. The form I and form II 2-μm DNA bands are discussed in the text.

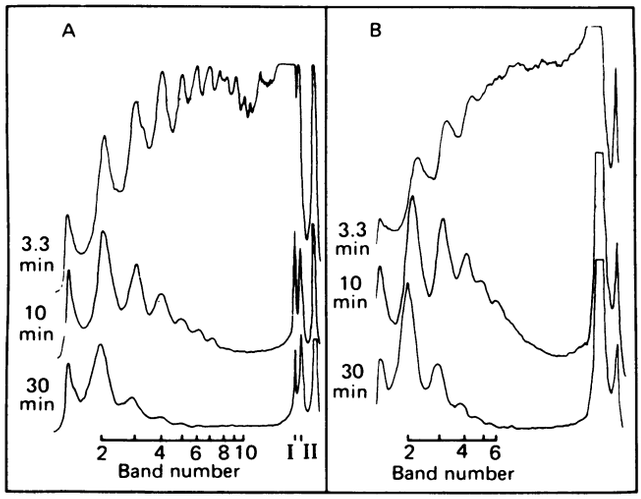

FIG. 2. Densitometer tracings of electrophoretic gel patterns. (*A*) Tracings of the 2-μm [^{32}P]DNA autoradiogram shown in Fig. 1*B*. (*B*) Tracings of lanes 1′, 2′, and 3′ of the chromosomal [^{32}P]DNA autoradiogram of Fig. 1*C*. The densitometer tracing of lane 1′ of the ethidium bromide-stained gel (Fig. 1*A*) from which these fragments were transferred reveals nucleosomal DNA fragments up to at least the decamer. The reason for the poor resolution of larger DNA fragments in the chromosomal DNA hybridization spectrum is unknown. Tracings were done with the Joyce–Lobel densitometer.

Table 1 summarizes the quantitative data obtained from the densitometric tracings. The average nucleosome repeat length (core plus spacer DNA) was determined for 2-μm chromatin and total chromatin in the same sample at different times of nuclease digestion. Repeat lengths were estimated by dividing the size of DNA in a band by the band number (dimer, trimer, etc.) and averaging this value for all the bands (different oligomers) present at that time of digestion. Essentially the same nucleosome repeat length was obtained for chromosomal DNA, at each digestion time, from the analysis of either ethidium bromide-stained gels or autoradiograms. This similarity indicates that the transfer and hybridization procedure does not significantly alter the digestion pattern. At the earliest digestion time (3.3 min) the measured repeat length for chromosomal DNA ranges from 163 to 169 base pairs. This value is similar to the 160- to 165-base pair repeat length determined by Lohr *et al.* (18). Throughout the course of digestion, the 2-μm nucleosome repeat lengths are not significantly different from those measured for total chromatin. We conclude that 2-μm DNA nucleosomes have the same repeat length as nucleosomes in total chromatin. Based on this repeat length, a 2-μm DNA molecule could accommodate 36–38 nucleosomes.

Sedimentation of Native 2-μm Plasmids. The minichromosome forms of polyoma and SV40 DNAs sediment in 0.15 M NaCl 3–4 times faster than the purified DNA molecules sediment (30–32). A form of 2-μm DNA with a similar sedimentation property is released from yeast spheroplasts upon osmotic lysis. After centrifuging lysed spheroplasts at low speed to pellet most of the nuclear chromatin, we examined the supernatant material by zone sedimentation in sucrose gradients containing 0.15 M NaCl. One sample of the supernatant was sedimented directly and a second sample was treated prior to sedimentation with 1% Sarkosyl to remove any noncovalently bound proteins. Each fraction from the two sucrose gradients was treated with Sarkosyl and subjected to gel electrophoresis. The 2-μm DNA was detected by autoradiography of the dried gel (Fig. 3). The sedimentation of 2-μm DNA in the untreated sample (Fig. 3*A*) was about 3 times faster than that of 2-μm DNA in the Sarkosyl-treated sample (Fig. 3*B*). The fast-sedimenting form is presumed to be the 2-μm minichromosome. The sedimentation constants for 2-μm DNA are 75 S and 24 S for the two conditions. By comparison, the SV40 minichromosome has a sedimentation constant of 70 S, whereas form I SV40 DNA, which is 10% smaller than 2-μm DNA, has a sedimentation constant of 21 S (30).

Both the Sarkosyl-treated and untreated samples contain both

Table 1. Nucleosome repeat lengths of total and 2-μm DNA chromatin

Detection method	Average nucleosome DNA repeat length, base pairs (SD)		
	3.3 min*	10 min*	30 min*
Ethidium bromide stain	163 (2.4)	154 (4.2)	145 (6.2)
Chromosomal [^{32}P]DNA probe	169 (3.8)	155 (3.5)	147 (3.3)
2-μm [^{32}P]DNA probe	165 (6.7)	161 (10.2)	147 (12.2)

* Digestion time.

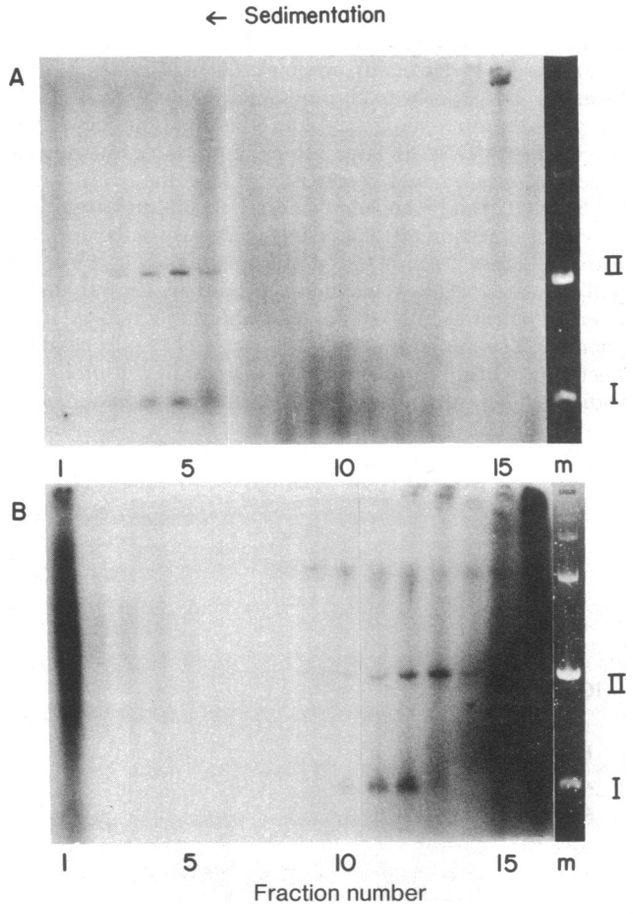

FIG. 3. Sedimentation analysis of 2-μm DNA released from lysed spheroplasts. ^{32}P-labeled spheroplasts of a 2-μm$^+$ strain were lysed by dilution into hypotonic buffer. After slow-speed centrifugation to pellet most of the cellular chromatin, the supernatant was analyzed by zone sedimentation through 5–20% sucrose gradients containing 0.15 M NaCl. Fractions from the gradients were treated with 1% Sarkosyl and loaded onto an agarose gel. Identical results were obtained when the hypotonic buffer contained Nonidet P-40. (*A*) Autoradiogram of fractions from a sucrose gradient in which the sample was sedimented directly. (*B*) Autoradiogram of fractions from a sucrose gradient in which the 2-μm-DNA-containing supernatant was made 1% in Sarkosyl before sedimentation. Lane m in both panels is a photograph of a lane in the same gels that contains superhelical (form I) and nicked (form II) 2-μm DNA standards. The lower-mobility band in *B* has not been identified.

form I and form II 2-μm DNA. In the untreated sample, the form I and form II DNAs have the same sedimentation constant (75 S) as shown by densitometric scans of the gel autoradiogram. This is analogous to SV40 minichromosomes (70 S), which also contain relaxed and covalently closed DNA. As expected, form I 2-μm DNA in the Sarkosyl-treated sample sedimented slightly faster (one gradient fraction) than form II DNA.

DISCUSSION

We have presented two results that indicate that the 2-μm DNA plasmid has a chromosome-like structure. First, discrete size classes of DNA fragments are produced from both 2-μm and chromosomal DNA by the digestion of total yeast chromatin with micrococcal nuclease. In both cases the sizes of the nuclease-resistant DNA fragments are integral multiples of the yeast nucleosome repeat length of 165 base pairs. The protected 2-μm DNA fragments must be derived from the free plasmids because no integrated 2-μm DNA sequences have been found in chromosomal DNA from either 2-μm^+ or 2-μm^- strains (6, 33). Second, 2-μm plasmids released from spheroplasts by osmotic lysis sediment at 75 S, about 3 times faster than plasmids treated with Sarkosyl prior to centrifugation. By analogy with the sedimentation constant of SV40 minichromosomes, 75 S is the approximate value expected for a 2-μm minichromosome. It is unlikely that 2-μm minichromosomes were created artifactually during the preparation procedures, because under the ionic conditions employed histone dissociation and exchange do not occur (34).

The results of several experiments (1, 9, 10) have suggested that 2-μm DNA molecules are located in the cytoplasm, possibly in an organelle. However, two properties of 2-μm DNA lead to the proposal that these molecules are present in the nucleus, at least during part of the cell cycle. First, we have shown here that 2-μm DNA has the same nucleosomal organization as chromosomal DNA. Other extrachromosomal DNAs that have a nucleosomal organization are localized in the nucleus. These DNAs include the ribosomal RNA genes of *Physarum* (35, 36) and *Tetrahymena* (37), the amplified ribosomal RNA genes of *Xenopus* (38) and *Dytiscus* (39), as well as the DNAs of SV40 and polyoma and Epstein–Barr viruses (40). Second, it is likely that 2-μm DNA replication takes place in the nucleus (13, 16). Mutations in *cdc* genes *4*, *7*, and *28*, which prevent nuclear DNA replication by blocking the entry of cells into the S phase (14, 15), also block 2-μm DNA replication (13) but not mitochondrial DNA replication (41). Moreover, it has recently been shown that 2-μm DNA replication is limited to a portion of the S phase, and that its replication is regulated such that each 2-μm DNA molecule is replicated once, and only once, each S phase (16). In contrast, mitochondrial DNA replication occurs throughout the cell cycle (42) and, at least in animal cells, proceeds by a mechanism in which some molecules replicate more than once and some not at all during a cell cycle (43).

A plausible explanation for these properties of 2-μm DNA is that during the S phase the molecules enter the nucleus where they replicate and simultaneously acquire a complement of nucleosomal proteins.

We thank Yves Mizrahi for technical assistance and Drs. Bonita Brewer, Breck Byers, and Virginia Zakian for criticism of the manuscript. This work was supported by grants from the National Institutes of Health and the American Cancer Society. R.G.N. was supported during a portion of this work by an American Heart Association of Washington Postdoctoral Fellowship.

1. Clark-Walker, G. D. & Miklos, G. L. (1974) *Eur. J. Biochem.* **41**, 359–365.
2. Beggs, J. D., Guerineau, M. & Atkins, J. F. (1976) *Mol. Gen. Genet.* **148**, 287–294.
3. Hollenberg, C. P., Degelmann, A., Kustermann-Kuhn, B. & Royer, H. D. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 2072–2076.
4. Guerineau, M., Grandchamp, C. & Slonimski, P. P. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 3030–3034.
5. Livingston, D. M. & Klein, H. L. (1977) *J. Bacteriol.* **129**, 472–481.
6. Cameron, J. R., Philippsen, P. & Davis, R. W. (1977) *Nucleic Acids Res.* **4**, 1429–1448.
7. Gubbins, E. J., Newlon, C. S., Kann, M. D. & Donelson, J. E. (1977) *Gene* **1**, 185–207.
8. Beggs, J. D. (1978) *Nature (London)* **275**, 104–109.
9. Livingston, D. M. (1977) *Genetics* **86**, 73–84.
10. Clark-Walker, G. D. (1972) *Proc. Natl. Acad. Sci. USA* **69**, 388–392.
11. Guerineau, M., Grandchamp, C., Pasletti, C. & Slonimski, P. P. (1971) *Biochem. Biophys. Res. Commun.* **42**, 550–557.
12. Broach, J. R., Atkins, J. F., McGill, C. & Chow, L. (1979) *Cell* **16**, 827–839.
13. Livingston, D. M. & Kupfer, D. M. (1977) *J. Mol. Biol.* **116**, 249–260.
14. Hartwell, L. (1973) *J. Bacteriol.* **115**, 966–974.
15. Hereford, L. & Hartwell, L. (1974) *J. Mol. Biol.* **84**, 445–461.
16. Zakian, V. A., Brewer, B. & Fangman, W. L. (1979) *Cell* **17**, 923–934.
17. Lohr, D. & Van Holde, K. E. (1975) *Science* **188**, 165–166.
18. Lohr, D., Kovacic, R. T. & Van Holde, K. E. (1977) *Biochemistry* **16**, 463–471.
19. Nelson, D. A., Beltz, W. R. & Rill, R. L. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 1343–1347.
20. Southern, E. M. (1975) *J. Mol. Biol.* **98**, 503–517.
21. Shelton, E. R., Wassarman, P. M. & DePamphilis, M. L. (1978) *J. Mol. Biol.* **125**, 491–514.
22. Forte, M. & Fangman, W. L. (1976) *Cell* **8**, 425–431.
23. Tabak, H. F. & Flavell, R. A. (1978) *Nucleic Acids Res.* **5**, 2321–2332.
24. Rigby, P. W. J., Dieckmann, M., Rhodes, C. & Berg, P. (1977) *J. Mol. Biol.* **113**, 737–751.
25. Mackey, J. K., Brackmann, K. H., Green, M. R. & Green, M. (1977) *Biochemistry* **16**, 4478–4483.
26. Denhardt, D. T. (1966) *Biochem. Biophys. Res. Commun.* **23**, 641–646.
27. Rubin, G. M. (1973) *J. Biol. Chem.* **248**, 3860–3875.
28. Piñon, R. & Salts, Y. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 2850–2855.
29. Freifelder, D. (1974) *J. Mol. Biol.* **54**, 567–577.
30. Keller, W., Muller, U., Eicken, I., Wendel, I. & Zentgraf, H. (1977) *Cold Spring Harbor Symp. Quant. Biol.* **42**, 227–243.
31. Griffith, J. D. & Christiansen, G. (1977) *Cold Spring Harbor Symp. Quant. Biol.* **42**, 215–226.
32. Hall, M., Meinke, W. & Goldstein, D. A. (1973) *J. Virol.* **12**, 901–908.
33. Tabak, H. F. (1977) *FEBS Lett.* **84**, 67–70.
34. Clark, R. J. & Felsenfeld, G. (1971) *Nature (London) New Biol.* **229**, 101–103.
35. Grainger, R. M. & Ogle, R. C. (1978) *Chromosoma* **65**, 115–126.
36. Seebeck, T., Stalder, J. & Braun, R. (1979) *Biochemistry* **18**, 484–490.
37. Mathis, D. & Gorovsky, M. A. (1976) *Biochemistry* **15**, 750–755.
38. Reeves, R. (1978) *Biochemistry* **17**, 4908–4916.
39. Scheer, U. & Zentgraf, H. (1978) *Chromosoma* **69**, 243–254.
40. Shaw, J. E., Levinger, L. F. & Carter, C. W., Jr. (1979) *J. Virol.* **29**, 657–665.
41. Newlon, C. S. & Fangman, W. L. (1975) *Cell* **5**, 423–428.
42. Williamson, D. H. & Moustacchi, E. (1971) *Biochem. Biophys. Res. Commun.* **42**, 195–201.
43. Bogenhagen, D. & Clayton, D. A. (1977) *Cell* **11**, 719–727.