Proc. Natl. Acad. Sci. USA
Vol. 74, No. 7, pp. 2850–2854, July 1977
Cell Biology

Isolation of folded chromosomes from the yeast *Saccharomyces cerevisiae*

(chromosome organization/supercoiled DNA)

R. PIÑON AND Y. SALTS

Department of Biology, University of California, San Diego, La Jolla, California 92093

Communicated by Dan L. Lindsley, March 17, 1977

ABSTRACT **Two fast-sedimenting chromatin complexes with sedimentation velocities of approximately 4600 and 3000 S can be isolated from logarithmically growing diploid *Saccharomyces cerevisiae* cells. The DNA in both structures appears to be folded into at least 60 domains and characterized by a negative superhelical density. Sensitivity to proteases and insensitivity to RNases suggest that proteins and not RNA are important in maintaining the organization of the chromosomes in both structures. The 4600S and 3000S complexes represent folded genomes isolated from diploid cells in the G2 and G1 stages of the cell cycle, respectively.**

Recent studies (1–3) have provided a rather consistent and unified picture of the subunit structure of chromatin as a flexible chain of spherical particles (nucleosomes), but information on how the nucleosomes are organized into higher order structures is still fragmentary. Although higher eukaryotic cell types provide suitable material for cytological or ultrastructural studies, it seems clear that at present the very large genomes of such cells will make it difficult to carry out well-defined physical and biochemical studies on the long-range features of chromosome organization. The microbial eukaryote, *Saccharomyces cerevisiae*, with a genome size about 3.3 times that of *Escherichia coli* (4), may provide a more amenable system for these types of studies. The work reported here indicates the feasibility of using the yeast system in studying the structural and functional aspects of chromosome organization in a eukaryotic cell.

We have found that the nuclear genome from logarithmically growing diploid cells can be isolated as two compact, fast-sedimenting chromosome complexes containing RNA, protein, and a trace of membrane components and characterized by sedimentation velocities of approximately 4600 S and 3000 S. The DNA in both structures is characterized by a negative superhelical density, as judged by sedimentation studies with ethidium bromide (EtBr). The DNA in each complex appears to exist in a folded form with many separate superhelical domains. We can estimate that there must be at least 60 such domains, and quite likely many more. Both structures are insensitive to pancreatic RNase A or T1 RNase, but are sensitive to proteases. Comparison of complexes from haploid, diploid, and tetraploid cells indicates that the 4600S and 3000S structures represent genomes isolated from diploid cells in the G2 and G1 stages, respectively.

MATERIALS AND METHODS

Strains and Growth Conditions. *Saccharomyces cerevisiae* strains X44 (*a*), 131 ($a\alpha$), and X212 ($aa\alpha\alpha$) were used in this study. X44 is the *a* parent of the diploid 131, and the tetraploid, X212, is a cross between strains 131-20-2 (*aa*) and 131-20-4 ($\alpha\alpha$), all described previously (5). All were grown in an acetate medium (PSP2) supplemented with 40 μg/ml of adenine, 10 μg/ml of uracil, and 40 μg/ml of leucine when required. Isotopic labeling of DNA was carried out by adding either 1–1.6 μCi/ml of [^{14}C]uracil (30 mCi/mmol, New England Nuclear) or 5 μCi/ml of [6-^{3}H]uracil (9.2 Ci/mmol, New England Nuclear) to PSP2 medium.

Preparation of Chromosome Complexes. Logarithmic (1 to 1.5 × 10^7 cells per ml) cultures of strain 131 (10–30 ml) were harvested by centrifugation (2 min at 1150 × *g*). After the cell pellet was washed twice in cold glass-distilled water, it was resuspended in 5 ml of 75 mM thioglycollate/0.1 M Tris·HCl at pH 8.8, made up just before use, and incubated for 15 min at 0°. The cells were then washed twice in cold glass-distilled water, resuspended in 2.5 ml of 1 M sorbitol and 2% (vol/vol) glusulase (Endo Laboratories), and incubated for 10–15 min at 37°. Spheroplast formation was monitored by phase microscopy. In all cases reported here, over 95% of the cells had been converted to spheroplasts by 10–15 min. The spheroplasts were washed by centrifugation (4 min at 1150 × *g*) three times with 5 ml of cold 1 M sorbitol. After each centrifugation the spheroplast pellet was resuspended very gently with a pasteur pipette. The last centrifugation was done in a small (10 × 75 mm) test tube. Spheroplasts were lysed by a modification of the method used by Hancock (6) in lysing cultured mouse cells. The spheroplast pellet was resuspended gently in 0.50 ml of LB buffer (1 mM KH_2PO_4/1 mM EDTA/0.2% Nonidet P-40 at pH 7.5), containing 25 mM 2-mercaptoethanol and 1 mM phenylmethylsulfonylfluoride, and allowed to sit at 0° for 30 min. Successful lysis was indicated by a clear, slightly opalescent lysate. To separate large debris we centrifuged the lysate for 3 min at 1000 × *g* at 4°. The resulting supernatant is referred to as the S fraction, and the pellet as the P fraction. An aliquot of the S fraction (0.4 ml) was then layered on a 12-ml 10–30% sucrose (density gradient sucrose, Mann) gradient containing 1 mM KH_2PO_4/1 mM EDTA at pH 8.5/25 mM 2-mercaptoethanol/1 mM phenylmethylsulfonylfluoride. Centrifugation was performed at 4° in an SW41 Beckman rotor at 5000 rpm for 35 min. This gradient was fractionated by piercing the bottom of the tube with a wide-bore (13 gauge) needle and allowing the solution to flow down the well walls of a tissue culture plate (Falcon, no. 3040). Fractions of approximately 0.3 ml were collected at a flow rate of about 1 ml/min. The tissue culture plate was kept in an ice bath during the fractionation of the gradient. The position of the chromosome complexes in the gradient was determined by spotting 20-μl aliquots from each fraction onto Schleicher and Schuell glass fiber filters. The filters were dried and radioactivity was determined in a toluene-based scintillation fluid containing 4.0 g of Omnifluor (New England Nuclear) per liter. Control experiments showed that the profile of the bottom three-quarters of the gradient looked

Abbreviations: EtBr, ethidium bromide; LB buffer, 1 mM KH_2PO_4/1 mM EDTA/0.2% Nonidet P-40 at pH 7.5; M_r, molecular weight.

the same whether total radioactivity or radioactivity in DNA was monitored. The three to four peak fractions of the faster sedimenting component (FI) and the more slowly moving component (FII) were pooled and to each were added 2 volumes of LB buffer. Both the diluted FI and FII fractions were stable when stored in the refrigerator for at least 3 days. Subsequent centrifugations of the diluted FI and FII fractions for the studies described below were performed by layering 0.2 ml of either fraction onto 5-ml 10–30% sucrose gradients containing 1 mM KH_2PO_4/1 mM EDTA at pH 8.5, and sedimenting at 4° for 35–40 min at 5000 rpm in an SW 50.1 Beckman rotor. These gradients were fractionated by dripping from the bottom and analyzing each fraction for alkali-stable, trichloroacetic acid-precipitable radioactivity as described (7).

The molecular weight of DNA from complexes FI and FII was determined by band sedimentation in sucrose gradients. Aliquots of freshly prepared undiluted FI or FII (0.1–0.2 ml) were layered on 5-ml 10–30% sucrose gradients. Neutral gradients contained 0.9 M NaCl/0.01 M EDTA/0.01 M Tris·HCl, pH 8.0/1.5% Sarkosyl; alkaline gradients contained 0.7 M NaCl/0.1 M NaOH/0.01 M EDTA. Centrifugation was performed in an SW 50.1 rotor for 22 hr at 11,000 rpm at 4°. As shown by Hutchinson and Krasin (8), under these centrifugation conditions molecules of molecular weight 200×16^6 or less are expected to show minimal rotor speed effects. ^{32}P-Labeled T4 DNA extracted from bacteriophage in 5% Sarkosyl/0.1 M EDTA at pH 8.0 at 60° (9) was used as molecular weight marker. Average molecular weights were computed from the relation $\overline{M}_r = \Sigma c_i m_i / \Sigma c_i$, where c_i is the counts per min in each fraction and m_i is the molecular weight corresponding to the fraction. Molecular weights were computed according to the relationships of Levin and Hutchinson (10, 11), using 1.1×10^8 and 5.5×10^7 as the molecular weights of double- and single-stranded T4 DNA, respectively.

EtBr Sedimentation Studies. Freshly prepared (less than 2 days old) FI and FII fractions containing about 0.1 μg of DNA were mixed with varying amounts of EtBr and layered on 5-ml sucrose gradients containing 1 mM KH_2PO_4/1 mM EDTA at pH 8.5 and corresponding concentrations of EtBr. The EtBr gradients were prepared in the dark, and the FI–EtBr and FII–EtBr mixtures were also kept in the dark. Centrifugation was as described above.

Electron Microscopy. Freshly prepared FI and FII components were spread for electron microscopy following the aqueous spreading procedure of Davis *et al.* (12). In many cases the FI or FII components were fixed in 1% formaldehyde/0.1 M KH_2PO_4, pH 7.5 for 5–10 min in an ice bath before they were mixed with ammonium acetate.

RESULTS

Lysis of spheroplasts in LB buffer results in a nonviscous, slightly opalescent lysate. Examination by phase-contrast microscopy shows that a few minutes after addition of LB buffer no intact spheroplasts or nuclei are left. Some debris remains, however, and this can be eliminated by the low-speed centrifugation. The supernatant from this low-speed centrifugation retains some 80% of the alkali-stable, trichloroacetic acid-precipitable radioactivity when [^3H]uracil or [^{14}C]uracil is used to label nucleic acids. Hence, only a small proportion of the DNA, and essentially all the debris, are removed by this preliminary centrifugation. Spheroplast lysis in the presence of divalent cations (5 mM Ca^{2+} or Mg^{2+}) or at high ionic strength (>0.2 M NaCl) is incomplete, with less than 50% of the spheroplasts lysing.

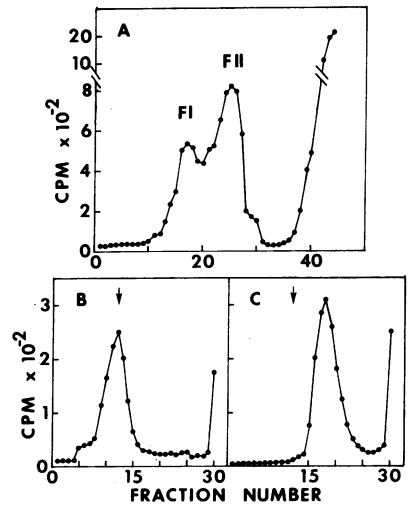

FIG. 1. Sedimentation profiles of the chromosome complexes. (*A*) Spheroplast lysate prepared from [^{14}C]uracil-labeled cells was placed on a 12-ml 10–30% sucrose gradient and centrifuged in a Beckman SW41 rotor at 5000 rpm for 35 min. The gradient was fractionated as described in *Materials and Methods* and 20-μl aliquots from each fraction were assayed for alkali-stable, trichloroacetic acid-precipitable radioactivity. (*B*) Fractions 16–18 (FI) from the gradient in *A* were pooled and diluted, and 0.2 ml of the diluted FI was run on a 5-ml 10–30% sucrose gradient in an SW 50.1 rotor at 5,000 rpm for 40 min. (*C*) Fractions 23–26 (FII) were pooled and diluted, and 0.2 ml of the diluted FII was centrifuged with the gradient in *B*. Arrows indicate the position of ^3H-labeled 4600S folded *E. coli* chromosomes that were attached to membranes (a gift from J. A. Silva) centrifuged in a parallel gradient. The direction of sedimentations is from right to left.

Characteristics of Chromosome Complexes. The characteristic feature of these lysates, as revealed by band sedimentation in sucrose, is the presence of two fast sedimenting components containing about 70% of the nuclear DNA in the gradient. Fig. 1*A* illustrates the sedimentation profile of alkali-stable, acid-precipitable radioactivity. We refer to the faster and more slowly sedimenting fractions as FI and FII, respectively. The sedimentation pattern is independent of the concentration of the lysate over at least a 30-fold range in concentration. Varying the concentration of the detergent Nonidet P-40 in LB buffer from 0.1 to 1% had no effect on the sedimentation pattern. The use of other detergents, 0.4% Tween 20 or Tween 80, 0.4% Triton X-100, or a mixture of 0.1% Brij-58 and 0.1% deoxycholate, also had no effect on the sedimentation pattern.

Addition of deproteinized yeast nuclear DNA to the LB lysate did not result in the cosedimentation of the exogenous DNA with either FI or FII. All of these results suggest that the high S values observed are probably not aggregation artifacts.

The relative proportions of FI and FII varied slightly from experiment to experiment, although in most cases FII represented about 60–70% of the total radioactivity. Both FI and FII, isolated from the preparative gradient as described in *Materials and Methods*, are stable for up to 5 days at 4°. The sedimentation profile of the isolated FI and FII components is shown in Fig. 1 *B* and *C*. FI is characterized by an S value of about 4600, and FII by an S value of about 3000. The S values were determined using the *E. coli* membrane-attached folded chromosome as a marker (13, 14). The S value of the *E. coli* chromosome (about 4600 S) was determined using T4 bacteriophage (1000 S) as a marker (15). The S value of the complexes remains constant between centrifugation speeds of 5000 and 14,000 rpm (when the centrifugal force × time of centrifuga-

tion is kept constant). Resedimentation of FI and FII during a 3-day period after their isolation resulted in invariant profiles and S values. Both FI and FII are stable for at least 30 min at 37°. The stability of both fractions appears to be due primarily to the presence of phenylmethylsulfonylfluoride in LB buffer. In the absence of phenylmethylsulfonylfluoride the complexes are stable for only a few hours.

Chemical determinations indicate that FI and FII contain, in addition to DNA, RNA, protein, and traces of phospholipid (less than 1% of the total mass). The weight ratio DNA:RNA: protein obtained from a number of different preparations is approximately 1:1:4. Both FI and FII precipitated in 0.1–0.2 M NaCl. At concentrations of NaCl greater than 0.5 M, the sedimentation velocity of FI and FII is reduced to less than 1000 S.

In the presence of 5 mM Mg^{2+} or Ca^{2+} the sedimentation velocity of both FI and FII increases significantly. With both Ca^{2+} and Mg^{2+}, the S value of FI increases from 4600 to over 7000 S. FII, in the presence of Mg^{2+}, goes from 3000 to about 4000 S, while with Ca^{2+} the increase is to about 6000 S. This effect is not reversible since the addition of EDTA after the Mg^{2+} and Ca^{2+} does not lead to a recovery of the normal FI and FII sedimentation profiles.

Both the FI and FII components contain endogenous RNase and proteolytic activity. The effect of the RNase activity is quite evident since the DNA:RNA ratio increases rapidly after isolation, and within 2 days most of the RNA has been lost. Quite significantly, however, the S values of FI and FII remain the same. We infer that a proteolytic activity is present because in the presence of the protease inhibitor (phenylmethylsulfonylfluoride) both FI and FII will remain stable for a much longer time than in the absence of phenylmethylsulfonylfluoride.

The regions of the gradient defined by FI and FII contain no detectable mitochondrial DNA, as judged by centrifuging FI and FII to equilibrium in CsCl. In contrast, mitochondrial DNA accounts for about 50% of the radioactivity in DNA in the fractions near the top of the gradient.

Superhelical Density of the DNA in FI and FII. The topological properties of the DNA in FI and FII were examined by the use of the intercalating dye, EtBr, in a manner similar to that used to study supercoiled molecules. For these studies freshly prepared FI and FII (less than 3 days old) complexes were used. The results of these EtBr titration experiments are summarized in Fig. 2. It is important to note that an untreated control gradient was always run in the same rotor with the EtBr-containing gradients and that the sedimentation patterns of both FI and FII are very reproducible. Hence, the changes noted are highly significant. Control experiments using ^{32}P-labeled T4 bacteriophage as a sedimentation marker showed that the S value of T4 phage did not vary with EtBr concentration, and hence, the EtBr-induced transitions are a property of the DNA in the chromosome complexes. The sedimentation coefficient at each concentration of EtBr has been normalized to the control value. As evident from Fig. 2, the sedimentation coefficient varies with EtBr in a manner that is similar to that of naturally occurring supercoiled DNA molecules. Both FI and FII follow essentially similar biphasic EtBr curves. Although the minimum S value is reached at 1–2 μg/ml of EtBr, values for which the superhelical density of circular λ and simian virus 40 DNA, as well as that of the folded chromosome of *E. coli*, approaches zero (16, 17), we cannot conclude that the superhelical density of the DNA in FI and FII is the same as in these other molecules. Under our low salt conditions, the binding affinity of EtBr to DNA would be expected to be greater than at the higher salt concentrations used in these other studies (18). Furthermore, the DNA in our complexes is not naked but is complexed with protein, and hence, the number of EtBr-binding sites is difficult to estimate. Nevertheless, it is clear from the EtBr data that the DNA in FI and FII is rotationally constrained, and that it is characterized by a negative superhelical density.

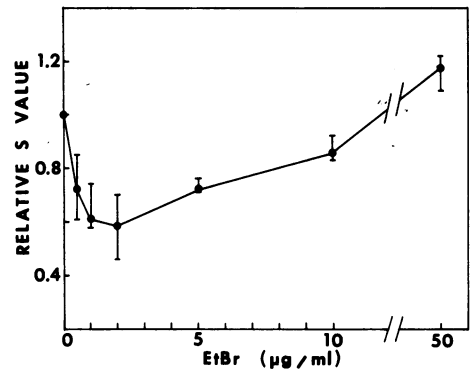

FIG. 2. Summary of EtBr sedimentation experiments. Centrifugation conditions were the same as in Fig. 1. The points represent results of one experiment. The error bars are two standard deviations about the mean of several experiments (five for 0.5 μg/ml, four for 1 μg/ml, nine for 2 μg/ml, three for 5 μg/ml, four for 10 μg/ml, and six for 50 μg/ml of EtBr). In each experiment the relative S value was derived from a comparison with a control gradient (no EtBr) run together with the EtBr-containing gradients.

Effects of DNase. The number of independent superhelical domains in a complex can be estimated by observing the reduced S values produced by a small number of DNA breaks. Both FI and FII are disrupted by extensive DNase cleavage with either DNase I or DNase II. However, brief treatment with these enzymes resulted in an *increase* in S value, although we ascertained that the DNA was broken by the enzymes. We attribute this anomalous behavior to aggregation caused by DNase II directly and by the divalent cations required with DNase I (see above). Nevertheless, a minimum estimate of the number of domains per yeast genome could be made. DNA in freshly prepared FI and FII complexes has a double-strand $\overline{M}_r$ of about 150×10^6, with a range from 100×10^6 to 200×10^6 (*Materials and Methods*); there are few single-strand nicks since the single-strand $\overline{M}_r$ is about half this value. Taking the genome size of yeast as 9×10^9 (4), and assuming the entire genome is rotationally constrained, then there are at least 60 superhelical domains. The actual number is probably much larger. Results with cells of different ploidy (below) are consistent with the idea that the entire genome is rotationally constrained in the complexes.

Effects of RNase and Protease. Neither FI nor FII was disrupted by T1 RNase, pancreatic RNase A, or endogenous RNase activity. Under incubation conditions where [^{32}P]RNA mixed with FI or FII was completely degraded to acid-soluble fragments, the characteristic S value of FI or FII did not change when FI or FII was incubated with T1 RNase. However, after treatment with RNase A, we observed an increase in the S value of both components. This is probably due to aggregation induced by RNase molecules binding to the DNA (19). In contrast, both components, FI and FII, are sensitive to proteases. With low levels of protease activity a spectrum of intermediate forms with decreasing S values is obtained, indicating that a more or less continuous change in conformation occurs with increasing amounts of protease. This indicates that there are probably many sites at which protein–protein or protein–DNA interactions are necessary to maintain the structural integrity

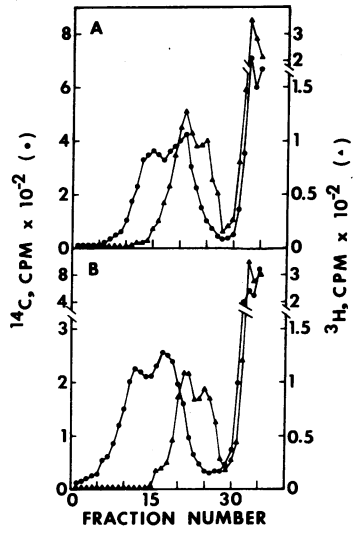

FIG. 3. Effect of ploidy on the sedimentation profile of the complexes. Cells from X44 (haploid) grown in PSP2 with 10 μCi/ml of [6-^{3}H]uracil were mixed with (*A*) cells of 131 (diploid) grown in the presence of 1 μCi/ml of [^{14}C]uracil and (*B*) cells of X212 grown in the presence of 1 μCi/ml of [^{14}C]uracil. Both cell mixtures were converted to spheroplasts, then lysed and loaded on 5-ml 10–30% sucrose gradients. Centrifugation conditions were 40 min at 5000 rpm in a Beckman SW 50.1 rotor. The direction of sedimentation is from right to left.

of the complexes. For levels of protease that do not dissociate the complex completely but that still result in a significant decrease in S value, the characteristic biphasic EtBr pattern is still obtained. This result argues again that the topological constraints imposed on the DNA result from protein–DNA interactions at many different sites.

FI and FII Are Functionally Different. The question of whether FI and FII represent structures isolated from cells at different stages in the cell cycle was examined by comparing the S values of complexes isolated from cells with different ploidy. Lysates from haploid and tetraploid strains showed that, as in diploids, fast sedimenting (FI) and slowly sedimenting (FII) components could be separated. However, the S values of the complexes isolated from each of the three different strains differed significantly (results not shown). The relationships between FI and FII from the three strains were clarified in the following experiment. Two cell mixtures were prepared, one containing ^{3}H-labeled haploid (X44) and ^{14}C-labeled diploid (131) cells, and the other, ^{3}H-labeled haploid (X44) and ^{14}C-labeled tetraploid (X212) cells. Spheroplasts from the two mixtures were prepared, lysed, and run on sucrose gradients. The results (Fig. 3) show that FI of the haploid cosediments with FII of the diploid, and FI of the diploid sediments with an S value similar to that of FII of the tetraploid. This correspondence indicates that FI and FII are structures isolated from cells in which the nucleus is in the G2 and G1 stages, respectively.

Electron Microscopy of the Chromosome Complexes. Electron microscopic visualization of components FI and FII by standard aqueous spreading techniques has revealed the types of structures shown in Fig. 4. These are typical for structures that have undergone mild fixation with formaldehyde before spreading. No obvious differences between the FI and FII structures have been noted. Features characteristic of these structures include a more or less condensed core with a complex array of DNA fibers extending outward. Under the standard high-salt spreading conditions it appears that most of the chromosomal proteins have been extracted, and the complex

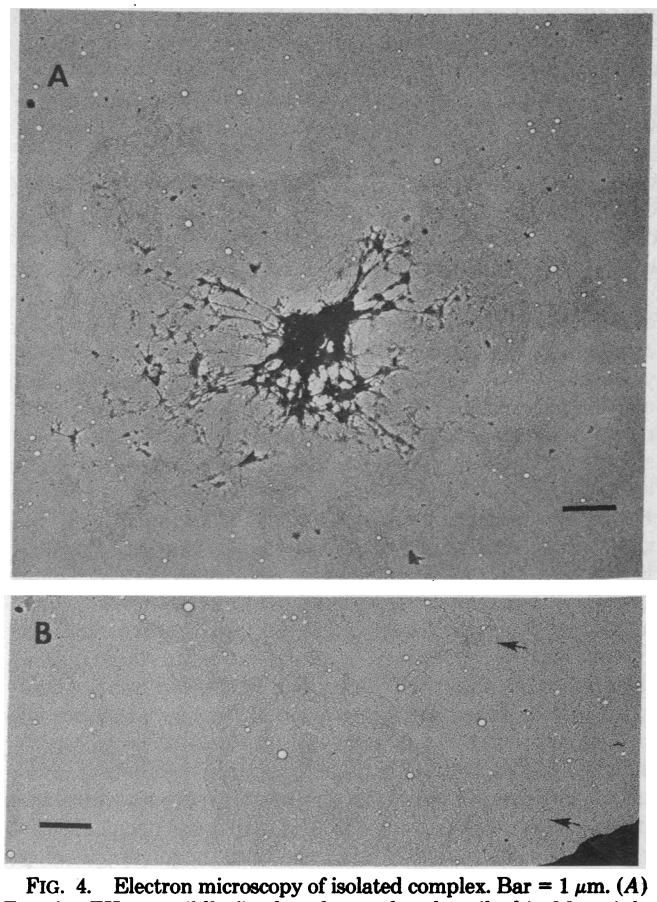

FIG. 4. Electron microscopy of isolated complex. Bar = 1 μm. (*A*) Fraction FII was mildly fixed, and spread as described in *Materials and Methods*. (*B*) Fraction FII was spread without prior fixation. Arrows indicate twisted DNA.

is greatly extended. The highly compact folding suggested by the sedimentation data has probably been lost upon spreading, although some remnants remain at the center of the complex. The electron-dense core at the center may include membrane components. In most cases where the fixation step was omitted, much more extended structures were seen, and as shown in Fig. 4*B*, clear evidence of supercoiling was visible, especially in the peripheral regions.

DISCUSSION

The studies described in this paper indicate that chromosomal DNA in logarithmically growing diploid *S. cerevisiae* cells is organized into two types of DNA–RNA–protein complexes characterized by sedimentation coefficients of about 4600 S and 3000 S. Together they account for at least 60–70% of the nuclear DNA in the cell. Although the insensitivity of both complexes to RNase indicates that RNA is probably not involved in stabilizing and maintaining the integrity of the complex, we cannot rule out the possibility that a "structural" RNA exists, which is either resistant or inaccessible to both exogenous and endogenous RNases. On the other hand, both the FI and FII structures are sensitive to proteases, which clearly indicates that proteins must play a crucial role in maintaining the conformational integrity of the complexes. Sedimentation studies with EtBr, supported by electron microscopic visualization, indicate that the DNA in the chromosome complexes is supercoiled. Although our data show that the superhelical density is negative, it has not been possible to calculate its value in a reliable way. Our data also suggest that the chromosomes in both FI and FII

are organized or folded into separate superhelical regions or domains. We can estimate that there must be at least 60 such domains in both FI and FII.

The subunit structure of yeast chromatin appears to be similar to that of higher eukaryotes (20) with nucleosomes of about 200 base pairs of DNA. Germond *et al.* (21) have shown that nucleosome formation generates supertwists in relaxed, closed circular simian virus 40 DNA. The supercoiled DNA we detect in our complexes is probably the result of disruption of the nucleosome structure of the chromatin. The DNA cannot relax due to protein–DNA interactions that keep it rotationally constrained in many independent folds. Lauer and Klotz (22), on the basis of viscoelastic retardation time measurements on yeast DNA, have suggested that the yeast genome may exist as one continuous DNA molecule, but at present the mitotic and meiotic behavior of yeast chromosomes seems most easily understood if there is a one-to-one correspondence between linear linkage groups and linear chromosomes. Comparison of FI and FII complexes from haploid, diploid, and tetraploid cells has shown that the difference in sedimentation between FI and FII is due primarily to differences in DNA content, and, consequently, it seems likely that FI and FII structures arise from G2 and G1 nuclei, respectively. Our ability to separate chromosome complexes from these two stages indicates that each complex must be intact to a large extent, that is, that the isolated complexes retain important aspects of their structure *in vivo*. We may imagine that the structure *in vivo* results from chromosomes being embedded in and attached to elements of an intranuclear protein matrix which provides the structural elements of the nucleus. Such a fibrous matrix has been described recently (23–25) and is felt to extend from the nuclear envelope into and throughout the interior of the nuclear volume. Such a matrix could provide the rotational constraints on the DNA implied by our data and the structural integrity for maintenance of nuclear shape after the nuclear envelope has been removed.

Note Added in Preparation. After submission of the manuscript for publication, we became aware of two articles in which the existence of superhelical DNAs from other eukaryotes was reported: an article by Cook and Brazell (26) (brought to our attention by one of the reviewers of the manuscript) on HeLa cells and one by Benyajati and Worcel (27) on cultured *Drosophila melanogaster* cells. Our study differs in that (*a*) we have been able to isolate the folded DNA complexes in a stable form so that they retain their structural integrity for a considerable period of time, (*b*) the two structures that we isolated appear to be intact and represent complexes from different phases of the cell cycle, and (*c*) preliminary evidence suggests that our structures contain all five histones, in agreement with the recent report that yeast chromatin contains all five histones (28) and some 30 other nonhistone proteins, as judged by two-dimensional gel electrophoresis.

We have not been able to obtain the FI and FII structures as described above from cells grown in glucose, a fact that we attribute to a different proteolytic activity not inhibited by phenylmethylsulfonylfluoride.

Thanks are due to R. Kavenoff for instructions in the art of electron microscopy, E. Leney, R. Timm, and J. Dorset for technical assistance during various phases of this work, and E. P. Geiduschek, B. Zimm, and H. Stern for their reading of the manuscript. This research was supported by a National Science Foundation Grant BMS 75-09.639, a CRCC Grant of the University of California to R.P., a Research Career Development Award to R.P., and an American Cancer Society California Division Postdoctoral Fellowship J-267 to Y.S.

The costs of publication of this article were defrayed in part by the payment of page charges from funds made available to support the research which is the subject of the article. This article must therefore be hereby marked "advertisement" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

1. Olins, A. L. & Olins, D. E. (1974) *Science* **183**, 330–332.
2. Kornberg, R. (1974) *Science* **184**, 868–871.
3. Oudet, P., Gross-Bellard, M. & Chambon, P. (1975) *Cell* **4**, 281–300.
4. Bicknell, J. N. & Douglas, H. C. (1970) *J. Bacteriol.* **101**, 505–512.
5. Simchen, G., Piñon, R. & Salts, Y. (1972) *Exp. Cell Res.* **75**, 207–218.
6. Hancock, R. (1974) *J. Mol. Biol.* **86**, 649–663.
7. Piñon, R., Salts, Y. & Simchen, G. (1974) *Exp. Cell Res.* **83**, 231–238.
8. Hutchinson, F. & Krasin, F. (1976) *Biophys. Chem.* **6**, 23–29.
9. Petes, T. & Fangman, W. L. (1972) *Proc. Natl. Acad. Sci. USA* **69**, 1188–1191.
10. Levin, D. & Hutchinson, F. (1973) *J. Mol. Biol.* **75**, 455–478.
11. Levin, D. & Hutchinson, F. (1973) *J. Mol. Biol.* **75**, 495–502.
12. Davis, R., Simon, M. & Davidson, N. (1971) in *Methods in Enzymology*, eds. Grossman, L. & Moldave, K. (Academic Press, New York), Vol. 21: part D, pp. 413–428.
13. Worcel, A. & Burgi, E. (1974) *J. Mol. Biol.* **82**, 91–105.
14. Ryder, O. A. & Smith, D. W. (1975) *Cell* **4**, 337–345.
15. Cummings, D. (1964) *Virology* **23**, 408–418.
16. Wang, J. C. (1969) *J. Mol. Biol.* **43**, 263–272.
17. Worcel, A. & Burgi, E. (1972) *J. Mol. Biol.* **71**, 127–147.
18. LePecq, J. B. & Paoletti, C. (1967) *J. Mol. Biol.* **27**, 87–107.
19. Sekine, H., Nakano, E. & Sakaguchi, K. (1969) *Biochim. Biophys. Acta* **174**, 202–210.
20. Lohr, D. & Van Holde, K. E. (1975) *Science* **188**, 165–166.
21. Germond, J. E., Hirt, B., Oudet, P., Gross-Bellard, M. & Chambon, P. (1976) *Proc. Natl. Acad. Sci. USA* **72**, 1843–1847.
22. Lauer, G. D. & Klotz, L. C. (1975) *J. Mol. Biol.* **95**, 309–326.
23. Aaronson, R. P. & Blobel, G. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 1007–1011.
24. Berezney, R. & Coffey, D. S. (1975) *Science* **189**, 291–293.
25. Keller, J. M. & Riley, D. E. (1976) *Science* **193**, 399–401.
26. Cook, P. R. & Brazell, I. A. (1975) *J. Cell Sci.* **19**, 261–279.
27. Benyajati, C. & Worcel, A. (1976) *Cell* **9**, 393–407.
28. Thomas, J. O. & Furber, V. (1976) *FEBS Lett.* **66**, 274–280.