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Isolation of an episomal yeast gene and replication origin as chromatin

(chromatin structure/linking number/electron microscopy/transcription/*Saccharomyces cerevisiae*)

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ABSTRACT **A multicopy yeast plasmid containing the *TRP1* gene (coding for *N*-5′-phosphoribosylanthranilate isomerase) and ARS*1* (autonomously replicating sequence *1*) has been purified as chromatin. Electrophoretic analysis of nucleic acid and proteins and electron microscopy show that the plasmid chromatin is largely free of contaminants. Electron-microscopic and linking-number analyses indicate that the plasmid chromatin contains seven nucleosomes, as predicted by the indirect end-label analyses of Thoma, Bergman, and Simpson [*J. Mol. Biol.* (1984) 177, 715–733]. Indirect end label mapping of micrococcal nuclease cuts demonstrates that nucleosome positions and nuclease-sensitive regions are not altered by the purification. The plasmid chromatin behaves homogeneously with respect to its elution from nuclei, template activity, and intrinsic buoyant density. Taken together, these observations suggest that different copies of the TRP1ARS1 plasmid do not differ from each other grossly in chromatin structure. We discuss the potential for understanding eukaryotic gene regulation offered by the ability to isolate unique genes as chromatin.**

Major advances have been made in recent years toward understanding DNA sequence elements that function as promotors and that are important in tissue or gene family-specific transcription in eukaryotes. These studies suggest, and results from classical genetic approaches in organisms such as yeast indicate, that transcriptional regulation involves trans-acting factors. Such factors have been partially or fully purified for a few genes (reviewed in ref. 1) by conventional techniques. A second promising approach to identify such factors is to assemble stable transcription complexes by adding specific DNA sequences to transcriptionally competent extracts and then to purify the complexes, taking advantage of their large size or precipitation behavior (ref. 2 and references therein). To date, neither of these approaches has been able to define an *in vitro* system that completely reflects the *in vivo* situation—that is, one that includes nucleosomes and other chromatin components as well as transcription factors.

With the aim of directly comparing the structure of a unique gene, as chromatin, in the active and inactive states (including identification of trans-acting regulatory factors), we have taken a different approach toward analysis of transcription and replication in eukaryotes. This is to purify the *in vivo* assembled chromatin of a specific gene. In principle, this approach eliminates the possibility of incomplete or incorrect assembly of a transcription complex *in vitro* and the need to identify and purify individually all of the components of the transcription complex. However, the difficulty of separating the chromatin of a single gene from all other chromatin and the need to use purification techniques that will maintain the integrity of a multisubunit complex have heretofore been formidable.

By using a single gene cloned into an autonomously replicating plasmid, we hoped to circumvent the difficulty of separating the chromatin of one gene from other chromatin. Further, by using a high-copy-number plasmid in an organism that can be grown readily in large amounts, it should be possible to obtain enough material for biochemical analyses. Our choice of the 1453-base-pair TRP1ARS1 [also called YARp1 (3)] plasmid of *Saccharomyces cerevisiae* as a model for developing a chromatin purification protocol was dictated by these considerations. The small size of the plasmid offers the additional advantage that regulatory factors might comprise a relatively large fraction of its total mass. Finally, the chromatin of this plasmid has been characterized by nuclease digestion studies (4); this provides a benchmark for assessing the effects of purification on the structural integrity of the plasmid chromatin. Here, we describe the complete purification of TRP1ARS1 plasmid chromatin. The methods described should permit one to purify chromatin containing other, regulated genes cloned into related plasmids.

MATERIALS AND METHODS

Isolation of Plasmid Chromatin. Buffers used consist of buffer A [80 mM KCl/5 mM $MgCl_2$/10 mM 2-(*N*-morpholino)ethanesulfonic acid, pH 6.3 with NaOH/1 mM EGTA/0.5 mM spermidine·HCl] and buffer B (200 mM NaCl/5 mM $MgCl_2$/10 mM Pipes, pH 7.3 with NaOH/0.5 mM EGTA) alone or in various combinations with the following additions: M (0.1% 2-mercaptoethanol), S (1 M sorbitol), F (18% Ficoll, average M_r = 400,000; Pharmacia), and I (1 mM phenylmethylsulfonyl fluoride/5 μg of pepstatin A per ml/1 mM iodoacetate, added freshly from 250×, 1000×, and 1000× stock solutions in ethanol).

SC3 cells (*Matα, ura3-52, his 3-1, trp1, gal2, gal10, cir°*) (5) containing the TRP1ARS1 plasmid, were grown at 30°C in 2% dextrose/0.67% nitrogen base without amino acids but supplemented with 0.002% each histidine and uracil to the start of a decline in growth rate. In our fermentors, where a pH of 5.5 was maintained by the addition of NH_4OH, this occurred at an OD_{600} of about 5. Cultures were cooled to room temperature and cells were harvested. Cells were suspended in 10 packed-cell volumes of buffer A containing M and were pelleted at 3500 rpm for 5 min in a JA10 rotor. They were then suspended in 5 cell volumes of buffer A containing S and M and incubated with 2 mg of zymolase 100,000 (Miles) per gm (wet weight) of cells at 30°C until the OD_{600} of the cells in 1% $NaDodSO_4$ was about 5% of the starting value. The suspen-

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Abbreviation: ARS, autonomously replicating sequence.

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sion was then diluted 1:1 (vol/vol) with buffer A containing S and pelleted as above, and the resultant spheroplasts were washed with 10 cell volumes of buffer A containing S and I. All subsequent procedures were carried out at 4°C.

Spheroplasts were suspended in 10 cell volumes of buffer A containing F and I and were stirred for 15 to 30 min. Lysis was completed by homogenization with a motor-driven Teflon pestle, and the lysate was centrifuged at 11,000 rpm for 20 min in a Sorvall GSA rotor. The pellet was suspended in 3 cell volumes of buffer A containing I, and 30- to 40-ml aliquots of the suspension were layered onto 150-ml gradients of 33% Percoll in buffer A containing S, preformed by centrifugation for 2 hr at 11,000 rpm in a GSA rotor. Loaded gradients were centrifuged in an HG4 rotor at 4400 rpm for 20 min. Nuclei in the upper of the two major bands were collected, diluted 1:2 with buffer A containing 0.1× I, pelleted by centrifugation for 5 min at 7500 rpm in a JA10 rotor, and washed with 2 cell volumes of buffer A with 0.1× I.

To elute plasmid chromatin, nuclei were incubated in 1/3 cell volume of buffer B containing M and 0.1× I minus iodoacetate and then were pelleted by centrifugation for 5 min at 7500 rpm in an HB4 rotor. This was repeated twice for a total elution time of 4 hr. The combined extracts were centrifuged at 11,000 rpm for 10 min in an HB4 rotor, loaded onto a 5 × 25 cm column of Sephacryl S-300, and eluted with buffer B containing M and 0.1× I minus iodoacetate. Material in the void volume was mixed with 2/3 volume of 100% Nycodenz (Nyegaard, Oslo) and centrifuged at 50,000 rpm in a VTi50 rotor for 36 hr. Gradient fractions containing plasmid chromatin were identified by gel electrophoresis and pooled. This pool was then centrifuged at 50,000 rpm in a VTi65.2 rotor for 18 hr as a second gradient. Fractions containing plasmid chromatin were again identified by gel electrophoresis and pooled. Nycodenz was removed by chromatography on Sephacryl S-300 in 0.6× buffer B containing M and 0.1× I minus iodoacetate. Material in the void volume was then layered onto linear 0.4–1 M sucrose gradients in 0.6× buffer B and centrifuged at 50,000 rpm in a VTi50 rotor for 80 min. Fractions containing plasmid chromatin were identified by gel electrophoresis and either frozen at −80°C or used immediately.

Protein Analysis. Yeast nuclear histones were obtained by acid extraction of nuclei, isolated as described above, and further purified by detergent washes and sedimentation through 2.7 M sorbitol. Proteins were electrophoresed on $NaDodSO_4$ gels (6) containing a 4% acrylamide stacking gel (acrylamide/methylenebisacrylamide = 30) and a resolving gel consisting of an 8–24% gradient of acrylamide (acrylamide/methylenebisacrylamide = 150).

Transcript Elongation Reactions. *In vivo*-initiated transcripts were elongated by mixing 0.1 mg of nuclei, plasmid chromatin containing nuclear extracts, or extracted nuclei, each in buffer B containing M and 0.1× I minus iodoacetate, with 0.1 ml of 20% glycerol/50 mM Hepes, pH 8.0 with NaOH/10 mM $MgCl_2$/1000 units of RNasin (Promega Biotec, Madison, WI) per ml/1 mM each ATP, CTP, and GTP/0.034 mM [α-^{32}P]UTP (75 Ci/mmol; 1 Ci = 37 GBq). After 30 min at 26°C, reactions were terminated by the addition of DNase I, followed by EDTA, $NaDodSO_4$, and proteinase K. RNA was further purified by extractions with phenol and chloroform, a second treatment with DNase I and proteinase K, and precipitations—once with 5% Cl_3CCOOH and twice with 0.9 M NH_4OAc and 66% ethanol. RNA was then incubated with an excess of nitrocellulose-bound *TRP1* DNA in 0.72 M NaCl/40 mM NaH_2PO_4/32 mM NaOH/4 mM EDTA/0.4% $NaDodSO_4$/2 mg of bovine serum albumin per ml at 70°C for 34 hr. Filters were washed as described (7) and assayed for radioactivity.

Nuclease Digestions and Nucleic Acid Analyses. Aliquots of DNA or chromatin in buffer B containing M were mixed with 1/20 volume of 0.1 M Hepes, pH 7.6 with NaOH/50 mM $MgCl_2$/50 mM $CaCl_2$ and incubated at 37°C with various amounts of micrococcal nuclease. Digestion was stopped by the addition of EDTA and proteinase K. After incubation at 37°C, 1/2 volume of 8.1 M NH_4OAc was added, and nucleic acids were precipitated with ethanol. For rapid analyses, pellets were suspended directly in gel loading buffer (6% glycerol/0.1% $NaDodSO_4$/25 mM Tris base/12.5 mM HOAc/12.5 mM Na_3EDTA/0.5 mg of bromophenol blue per ml) and heated at 60°C prior to loading. In other cases, DNA was further purified by treatment with RNase A and extraction with phenol and chloroform. Restriction enzymes from either Bethesda Research Laboratories or New England Biolabs and calf thymus topoisomerase I from Bethesda Research Laboratories were used under conditions recommended by the suppliers. DNA topoisomers were separated in composite 0.5% agarose/2% polyacrylamide gels run at 4°C in 36 mM Tris/30 mM NaH_2PO_4/1 mM EDTA (8). Other DNAs were fractionated in agarose gels in 50 mM Tris/25 mM HOAc/1 mM EDTA. Blotting and hybridizations were described (7). Probes were isolated and labeled by standard methods (9).

Electron Microscopy. Chromatin was prepared for electron microscopy by dialysis against 20 mM NaCl/10 mM triethanolamine·HCl, pH 8.0/1 mM Na_3EDTA and then against the same buffer containing 0.1% glutaraldehyde for 12–18 hr (10). Fixed chromatin was diluted into 10 mM NaCl/10 mM triethanolamine·HCl, pH 8.0/1 mM Na_3EDTA/0.0002% benzyldimethylalkylammonium chloride to a DNA concentration of about 1 μg/ml and was adsorbed onto carbon-coated alcian blue-treated grids (11). Grids were stained in 1% uranyl acetate, platinum-shadowed at an angle of 15 degrees, and examined in a Phillips 300 microscope at 80 KeV.

RESULTS

Purification of Plasmid Chromatin. Up to 10% of the mass of growing yeast is nucleic acid (12), and about 2% of yeast nucleic acid is DNA (13). The TRP1ARS1 plasmid comprises about 1% of the total DNA in the strain used here (ref. 3; see *Materials and Methods*). Thus, purification of plasmid chromatin requires, roughly, a 5000-fold enrichment with respect to total nucleic acids. To effect such a purification, we exploited a number of properties of the plasmid chromatin, the first being its nuclear location. Plasmid chromatin was eluted from purified nuclei in a buffer that represents a compromise among three goals. First, Mg^{2+} was present to keep residual ribosomes intact, which simplifies their later removal. Second, ionic strength was kept relatively low to minimize loss of chromatin proteins and dissociation of nucleosomes. Third, detergents were not used, thereby avoiding lysis of residual mitochondria in the nuclear pellet. Elution of most or all of the plasmid chromatin followed an apparent first-order time course (not shown), suggesting that elution was a diffusion-limited process. Plasmid chromatin was eluted slowly, even when nuclei were held in spheroplast lysis buffer A, so that the speed with which nuclei could be isolated affected final yields.

This monophasic elution pattern suggests that most copies of the plasmid chromatin are structurally similar and not bound to a nuclear structure that is stable in the eluting buffer. Nonetheless, it remains possible that a fraction of the plasmid chromatin is in a different form and essentially not elutable. This is of concern when working with a multicopy gene, since not all copies of the gene need to be in the same functional state, and a subset of plasmid chromatin could be lost during purification. To examine this possibility, nuclei were incubated in elution buffer until 80% of the plasmid chromatin had eluted. Residual nuclei and the eluate were then treated with micrococcal nuclease and the pattern of cuts on TRP1ARS1 DNA in both fractions was examined. While a portion (about half) of the noneluted plasmid chro-

matin resisted digestion, the nucleosome-like pattern of the digested portion was similar to that of the eluted fraction (Fig. 1). This suggests that <10% of the plasmid chromatin can be in a form different from the remainder. The nucleosome-free regions 5′ and 3′ to the *TRP1* gene and nucleosome positions in the nontranscribed portion of the plasmid were examined by indirect end label analyses (Fig. 2). These revealed minor differences between the eluted and noneluted fractions: cuts prominent in digests of naked DNA were more evident in the eluted fraction than in the noneluted fraction (compare peak 5 in "DNA," "extract," and "retained" profiles in Fig. 2) or in previously analyzed material (4). Possibly a portion of the eluted fraction was partially stripped of histones. Except for this difference, TRP1ARS1 chromatin in both eluted and noneluted fractions appears to have a structure virtually identical to that determined by Thoma *et al.* (4).

To examine the possibility that only the eluted or noneluted plasmid fraction is transcriptionally active, nuclei were incubated in elution buffer until about 75% of the plasmid chromatin had been eluted. The extract and residual nuclei were then incubated separately in a transcript elongation buffer, and RNA was isolated. *TRP1*-hybridizing RNA was 3-fold more abundant in the extract than in the residual nuclei, roughly proportional to the template concentration. The amount of *TRP1*-hybridizing RNA from the extract and residual nuclei together equalled the amount obtained from an equivalent number of nonextracted nuclei. While this result does not prove that each plasmid molecule is transcriptionally active, it indicates that preparation of nuclear extracts does not separate active and inactive forms of the plasmid chromatin.

After removing low molecular mass components from the nuclear eluate by chromatography on Sephacryl S-300, plasmid chromatin was banded isopycnically twice in Nycodenz density gradients. This effects a substantial purification and serves to concentrate the chromatin. The chromatin bands in a fairly symmetric peak at about 1.2 gm/ml. Material from either side of the peak rebanded with an average density of 1.2 gm/ml. In addition, chromatin from the low- and high-density sides of the peak appeared to have a pattern of micrococcal nuclease sensitivities similar to each other and to that of the starting material (not shown), indicating that the density gradient did not separate structural variants of the plasmid.

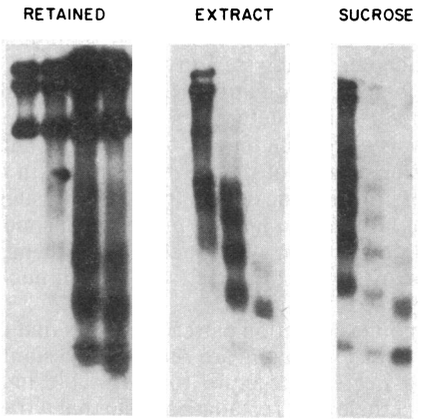

FIG. 1. TRP1ARS1 plasmid chromatin at different stages of purification. Aliquots of TRP1ARS1 chromatin remaining in nuclei after extraction ("retained"), in the nuclear extract ("extract"), and after final purification in sucrose gradients ("sucrose") were incubated with micrococcal nuclease for 10, 50, 250, and (for "retained") 1250 units·min·ml^{-1}. DNA from these digests was fractionated, blotted to nitrocellulose, and hybridized to a TRP1ARS1 probe. The highest mobility band in each panel corresponds in size to mononucleosomal DNA. The three lowest mobility bands, most prominent in "retained," are forms II, III, and I DNA.

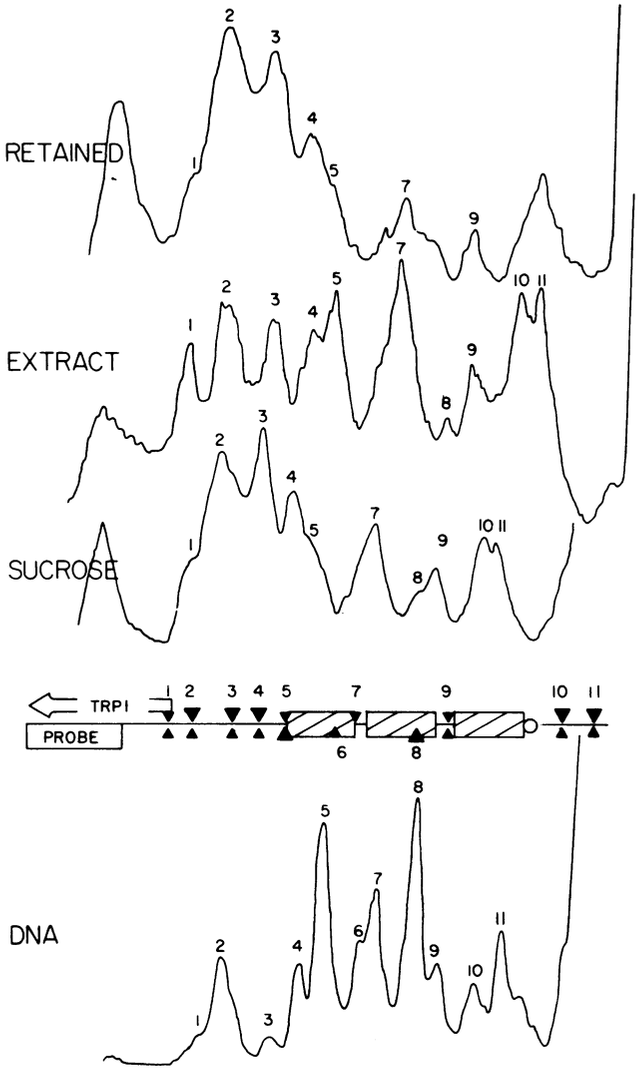

FIG. 2. Indirect end-label mapping (14, 15) of micrococcal nuclease cuts made on plasmid chromatin from different stages of purification. DNA from the digests described in Fig. 1 and from micrococcal nuclease digests of naked TRP1ARS1 DNA was cut with *Eco*RV, fractionated, blotted to nitrocellulose, and hybridized with a probe that abuts the *Eco*RV site. The resulting autoradiograms were scanned, and representative densitometric tracings from each substrate are shown. Numbers indicate correspondences among peaks in different scans and with cut sites shown as triangles in the drawing. Large triangles are strong cut sites, while small triangles are weak ones. Triangles above the map occur in chromatin, while those below are found in protein-free DNA. Hatched boxes indicate probable nucleosome positions inferred from occlusion of naked DNA cuts in chromatin substrates. Cuts were mapped by using internal length standards obtained from restriction digests of TRP1ARS1 DNA and are in good agreement with those mapped by Thoma *et al.* (4). The open circle in the drawing indicates the location of the core ARS*1* sequence.

The final purification step entails sedimentation in a linear sucrose gradient. From rotor geometry and sedimentation of yeast ribosomes in similar gradients, we calculate an *S* value of about 30 for TRP1ARS1 plasmid chromatin. Fig. 3 shows an ethidium bromide-stained gel of total nucleic acid from such a sucrose gradient; the plasmid DNA is largely supercoiled, and there is no other discrete nucleic acid species cosedimenting with the plasmid chromatin. In many preparations, some of the chromatin sedimented more slowly (evident in Fig. 3), suggesting that it had been partially stripped of histones, a phenomenon which has been shown to

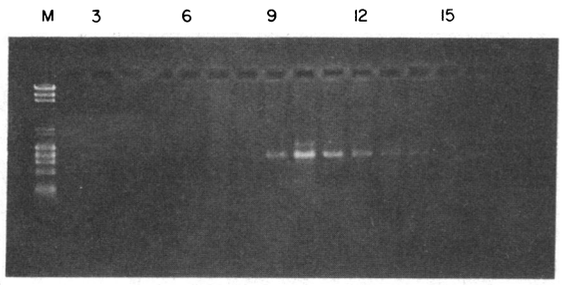

FIG. 3. Sucrose gradient fractionation of TRP1ARS1 plasmid chromatin. Sucrose gradients containing plasmid chromatin were collected in 20 fractions, and total nucleic acid from fractions 2 through 17 was electrophoresed on an agarose gel and stained with ethidium bromide. Sedimentation was from right to left. Gel markers (lane M) consist of phage λ and φX174 DNAs cut with *Hin*dIII and *Hae* III, respectively. Most of the plasmid DNA migrates as form I DNA, indicating that it survives purification intact. The slower-migrating band visible only in lanes 10 and 11 is form II DNA.

occur at high dilutions of chromatin (refs. 16 and 17; R.T.S., unpublished observations).

Structure of the Purified Plasmid Chromatin. Plasmid chromatin from the 30S region of sucrose gradients was digested with micrococcal nuclease, and the pattern of cuts was examined by both direct hybridization (Fig. 1) and by indirect end-labeling (Fig. 2). The patterns are similar to those seen in less-purified preparations, indicating that both nucleosomes and the structures seen as nuclease hypersensitive sites were intact. Cuts prominent in digests of naked DNA were less prominent in chromatin from the sucrose gradient than in the nuclear extract fraction (compare peak 5 in "DNA," "sucrose," and "extract" profiles in Fig. 2), again suggesting that partially or fully stripped plasmid chromatin sediments more slowly in the sucrose gradient and is excluded from the analysis.

Electron micrographs of two fields of plasmid chromatin are shown in Fig. 4. Most molecules appear as circles with up to seven beads, which we interpret to be nucleosomes. However, some appear to have fewer than seven nucleosomes or to be nucleosome free, consistent with the notion that nucleosome dissociation sometimes occurs. The number of nucleosomes per molecule is displayed in a histogram (Fig. 4) showing that the distribution of nucleosomes among different molecules is quite uniform.

We recently determined that the linking-number change per core particle is −1.0 (18), as previously suggested by others (8, 19). Purified TRP1ARS1 plasmid DNA was electrophoresed on a gel that resolves topoisomers (Fig. 5). Most of the molecules had a linking-number change of −6 to −8 relative to relaxed circles. The average linking-number change of −7 is consistent with TRP1ARS1 DNA being packaged as a complex of seven nucleosomes. Thus, nuclease digestions and electron-microscopic and linking-number analyses all gave an internally consistent picture of the plasmid chromatin structure.

Total protein from purified plasmid chromatin, and histones purified from yeast nuclei, were electrophoresed on gradient $NaDodSO_4$/acrylamide gels and stained first with Coomassie blue and then with silver (Fig. 6). Clearly, silver stained nonhistone proteins in the histone preparation more intensely than the histones. That even with silver staining histones appeared to be the major protein constituents of plasmid chromatin attests to the purity of the preparation. In addition to histones, the plasmid chromatin contained four proteins ranging from about 56 to 82 kDa, one of which (66 kDa) is visible in the Coomassie stained gel. A number of other proteins were present at lower (probably substoichiometric) abundance. These could be poorly staining species,

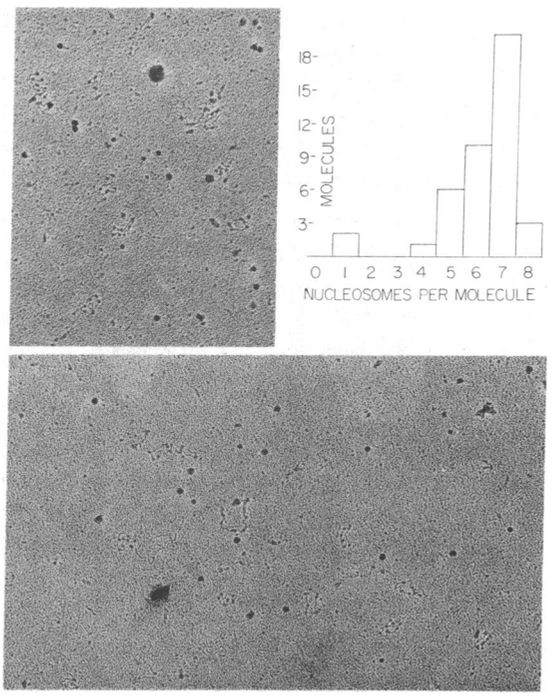

FIG. 4. Electron microscopy of purified TRP1ARS1 plasmid chromatin. Molecules of plasmid chromatin from sucrose gradients were visualized by electron microscopy. We assume that beaded structures along the DNA path are nucleosomes. The large opaque spots are due to residual Nycodenz. Nucleosomes on 42 molecules where the path of the DNA could be traced were counted, and these data are displayed on a histogram.

proteins present only in a subset of the plasmid chromatin molecules, proteolytic breakdown products of high molecular mass chromatin constituents, or nonchromatin contaminants. At present, it is not possible to distinguish among these possibilities.

Purity of Isolated Plasmid Chromatin. Purity of the plasmid chromatin was assessed by several means. Cumulative enrichment with respect to total nucleic acid was estimated at several steps in the purification and, after the final step, was close to the calculated enrichment required for complete purity (5000-fold). Both the small number of proteins and the

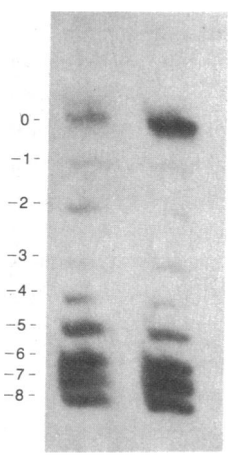

FIG. 5. Linking-number analysis of TRP1ARS1 DNA. DNA was either treated (right lane) or not treated (left lane) with calf thymus topoisomerase I, fractionated, partially hydrolyzed, denatured, blotted to GeneScreenPlus (New England Nuclear) and hybridized with a TRP1ARS1 probe. Numbers indicate linking-number changes relative to relaxed closed circular DNA.

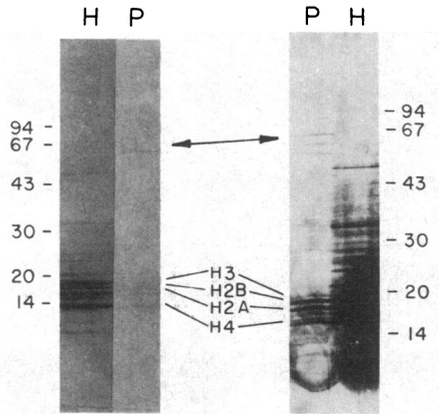

FIG. 6. Proteins of the purified TRP1ARS1 plasmid chromatin. Total plasmid protein (P) and yeast histones (H) were electrophoresed on an 8–24% gradient $NaDodSO_4$/polyacrylamide gel. The mobility of molecular weight markers is indicated in kDa. The gel was first stained with Coomassie blue (*Left*). The barely visible 66-kDa protein and histones from the plasmid chromatin are indicated. After extensive destaining in 50% methanol, the gel was stained with silver (20) (*Right*).

prominence of the histones among those proteins suggests that the preparation was nearly pure with respect to protein. Because most of the plasmid DNA survived the purification intact, any contaminating genomic or mitochondrial DNA also should be relatively intact and evident as discrete low mobility bands in Fig. 3. No such bands are evident. Thus, such contaminants make no significant contribution to the protein pattern in Fig. 6. Possible contamination by ribosome subunits is argued against by lack of visible rRNA in Fig. 3 and by the absence of ribosomal proteins in Fig. 6. Thus, we judge the purity and overall yield (about 10%) achieved by this procedure as sufficient to permit biochemical characterizations of the chromatin of a unique gene.

DISCUSSION

We describe the purification of a single eukaryotic gene as chromatin. Electron microscopic, compositional, and nuclease susceptibility data are all consistent with retention of the plasmid's *in vivo* state throughout the purification. This procedure offers the potential to clone chromatin (as opposed to cloning genes) and to compare, for instance, proteins associated with a single regulatable gene before and after induction of transcription. In this regard, possible heterogeneity of the material is significant. For example, a growing body of evidence suggests that transcribed chromatin may be preferentially associated with the nuclear matrix (ref. 21 and references therein). Although TRP1ARS1 plasmid chromatin is fairly refractile to elution in some buffers, possibly reflecting association with a nuclear structure, both the elution kinetics and a comparison of eluted and noneluted fractions by transcript elongation and micrococcal nuclease digestion suggests that the plasmid chromatin purification does not result in the exclusion of functional or structural variants.

In this study, we have isolated, as chromatin, a segment of yeast DNA and related its composition and structure to the structure inferred from earlier indirect end-label mapping (4). Indirect end-label mapping of micrococcal nuclease cuts in chromatin has been used in a number of studies in order to localize nucleosomes. All of these have used an often tacit, operational definition for the presence of a nucleosome—namely, that a region of >140 base pairs of DNA protected from digestion reflects the presence of a histone octomer bound to DNA. This report supports the use of this operational definition: direct morphological observations of the purified material and analysis of linking-number change indicate that the TRP1ARS1 plasmid chromatin contains seven nucleosomes, in agreement with the model based on our previous micrococcal nuclease mapping data (4). Our results are not consistent with a recently proposed alternative model of TRP1ARS1 chromatin structure (22).

The concentration of the single-copy chromosomal *TRP1* gene in a yeast nucleus is roughly 1 nM. During purification, the TRP1ARS1 plasmid chromatin concentration is of the same order. Thus, it should be possible to recover sequence-specific binding factors by the methods presented here. Whether any of the nonhistone proteins visible in Fig. 6 is such a protein is not known. We speculate that one or more of these nonhistone proteins may be associated with nuclease-sensitive regions at the 5′ end of the *TRP1* gene and in the *ARS1* region (23–25) of the plasmid chromatin, since these structures are preserved through the isolation. Since 5′ nuclease-sensitive regions may reflect association with transcription factors (reviewed in ref. 26), any protein associated with the nuclease-sensitive region 5′ to the *TRP1* gene on the plasmid chromatin might be a transcription factor.

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1. Dynan, W. S. & Tjian, R. (1985) *Nature (London)* **316,** 774–778.
2. Culotta, V. C., Wides, R. J. & Sollner-Webb, B. (1985) *Mol. Cell. Biol.* **5,** 1582–1590.
3. Zakian, V. A. & Scott, J. F. (1982) *Mol. Cell. Biol.* **2,** 221–232.
4. Thoma, F., Bergman, L. W. & Simpson, R. T. (1984) *J. Mol. Biol.* **177,** 715–733.
5. Sigurdson, D. C., Gaarder, M. E. & Livingston, D. M. (1981) *Mol. Gen. Genet.* **183,** 59–65.
6. Laemmli, U. K. (1970) *Nature (London)* **227,** 680–685.
7. Pederson, D. S., Yao, M.-C., Kimmel, A. R. & Gorovsky, M. A. (1984) *Nucleic Acids Res.* **12,** 3003–3021.
8. Germond, J. E., Hirt, B., Oudet, P., Gross-Bellard, M. & Chambon, P. (1975) *Proc. Natl. Acad. Sci. USA* **72,** 1843–1847.
9. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).
10. Thoma, F., Koller, Th. & Klug, A. (1979) *J. Cell Biol.* **83,** 403–427.
11. Labhart, P. & Koller, Th. (1981) *Eur. J. Cell. Biol.* **24,** 309–316.
12. Stewart, P. R. (1975) in *Methods in Cell Biology*, ed., Prescott, D. M. (Academic, New York), Vol. 12, pp. 111–147.
13. Potashkin, J. A., Zeigel, R. F. & Huberman, J. A. (1984) *Exp. Cell Res.* **153,** 374–388.
14. Nedospasov, S. A. & Georgiev, G. P. (1980) *Biochem. Biophys. Res. Commun.* **92,** 532–539.
15. Wu, C. (1984) *Nature (London)* **309,** 229–235.
16. Cotton, R. W. & Hamkalo, B. A. (1981) *Nucleic Acids Res.* **9,** 445–457.
17. Ausio, J., Seger, D. & Eisenberg, H. (1984) *J. Mol. Biol.* **176,** 77–104.
18. Simpson, R. T., Thoma, T. & Brubaker, J. M. (1985) *Cell* **42,** 799–808.
19. Keller, W. (1975) *Proc. Natl. Acad. Sci. USA* **72,** 4876–4880.
20. Wray, W., Boulikas, T., Wray, V. & Hancock, R. (1981) *Anal. Biochem.* **118,** 197–203.
21. Jackson, D. A. & Cook, P. R. (1985) *EMBO J.* **4,** 919–925.
22. Long, C. M., Brajkovich, C. M. & Scott, J. F. (1985) *Mol. Cell. Biol.* **5,** 3124–3130.
23. Tschumper, G. & Carbon, J. (1980) *Gene* **10,** 157–166.
24. Celniker, S. E., Sweder, K., Srienc, F., Bailey, J. E. & Campbell, J. L. (1984) *Mol. Cell. Biol.* **4,** 2455–2466.
25. Srienc, F., Bailey, J. E. & Campbell, J. L. (1985) *Mol. Cell. Biol.* **5,** 1676–1684.
26. Pederson, D. S., Thoma, F. & Simpson, R. T. (1986) *Annu. Rev. Cell Biol.* **2,** 117–147.