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The organization of oligonucleosomes in yeast

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ABSTRACT

We have developed a method of preparing yeast chromatin that facilitates the analysis of nucleoprotein organization. Yeast chromatin, isolated as an insoluble complex, is digested with micrococcal nuclease and fractionated into major insoluble and soluble fractions. No nucleosomal repeat is seen early in digestion for the insoluble fraction. Nucleosomal complexes of the soluble fraction are excised by nuclease in a distinctive non-random pattern; they are markedly depleted in mononucleosomes. When we analyze the soluble material by high resolution native electrophoresis, we find that the nucleoproteins resolve into two bands for each DNA multimer of the nucleosomal repeat. Our results suggest that there are structural similarities between bulk yeast chromatin and chromatin configurations found in transcribing genes of complex eukaryotes.

INTRODUCTION

The yeast Saccharomyces cerevisiae is an attractive organism for chromatin research because it is a simple, transcriptionally active eukaryote for which powerful biochemical and genetic techniques are available. Present knowledge of yeast chromatin derives principally from two experimental approaches; [1] Digestion of chromatin or nuclear preparations with micrococcal nuclease (1-5) or DNase I (3-7), followed by gel electrophoresis of purified total DNA. The electrophoresed DNA may subsequently be transferred to a blot, and probed with defined DNA or RNA sequences (3-6). [2] Chemical extraction of proteins from chromatin or nuclei, followed by physical studies (8,9) or characterization of post-translational modifications (10,11).

Little direct information exists on the heterogeneity and arrangement of nucleoprotein complexes in yeast chromatin, primarily because it is difficult to prepare intact chromatin nucleoproteins. Studies on isolated nucleoprotein fractions indicate that these complexes have a typical complement of core histones, but suggest that the mononucleosome may be

unstable upon isolation (12,13).

We report here methods that permit a direct assessment of heterogeneity among soluble yeast nucleoproteins by electrophoresis on a high resolution native gel. We have specifically examined the compositional and structural heterogeneity of mononucleosomes and short oligonucleosomes. We have also examined by gel electrophoresis the DNA patterns of chromatin that has been fractionated into soluble and insoluble portions after digestion with micrococcal nuclease.

Our results indicate that bulk yeast chromatin differs markedly in certain structural features from the bulk chromatin of higher eukaryotes. Comparison of our results to recent work on active metazoan genes suggests that yeast chromatin may have a general physical structure characteristic of transcriptionally competent chromatin in complex organisms.

MATERIALS AND METHODS

Conversion of yeast to mitochondrial DNA-free strain.

Saccharomyces cerevisiae, strain Y-55, was converted to the petite phenotype ρ^0 by growing the yeast for 24 hr at 30° in YEPD medium containing 10 µg/ml ethidium bromide (14), and using this culture as an inoculum for a further 24 hr growth in fresh medium under identical conditions.

Growth and spheroplasting of yeast.

The Y-55 ρ^0 strain was grown on YEPD medium at 30° to a logarithmic growth phase density of about 5 x 10^7 cells/ml, and the culture was immediately made 0.5 mM in PMSF. Cells were harvested by centrifugation for 5 min at 5,000 g, and subsequently washed twice in cold distilled H_2O containing 0.5 mM PMSF and once in S_1 buffer (1.4 M Sorbitol, 40 mM Na-HEPES, 0.5 mM $MgCl_2$, 1.0 mM PMSF, pH 7.5). Five liters of culture gave a cell pellet of about 12 grams wet weight.

The spheroplasting enzyme, lyticase (fraction II), was prepared according to Scott and Schekman (15), using a glucan substrate derived from three alkaline extractions of yeast cells. Lyticase stored at 4° remained active for two years. Washed cells were suspended in S_1 buffer (4 ml/gram cells) containing 0.5% β-mercaptoethanol. An empirically determined volume of lyticase, sufficient to completely convert cells to spheroplasts in about 90 min, was diluted with an equal volume of 2X S_1 buffer plus β-mercaptoethanol, readjusted to pH 7.5, and then added to

the cell suspension. Conversion to spheroplasts was at 30° with gentle agitation. Completion was assayed by subjecting a small drop of the suspension to gentle manual shearing between a microscope coverslip and slide, and visually determining that little or no cell wall remained in the lysate.

Isolation of nuclei and preparation of insoluble chromatin.

The isolation protocol incorporated elements of the methods of May, and of Bhargava and Halvorson as modified by Sajdel-Sulkowska et al. (16-18). All steps were on ice or at 4°; centrifugation steps were in a Sorvall HB-4 swinging bucket rotor. Upon conversion to spheroplasts, the cell suspension was diluted by the addition of 2-3 volumes of S_2 buffer (1.4 M Sorbitol, 20 mM Na-PIPES, 0.5 mM $MgCl_2$, 0.5 mM PMSF, pH 6.5) to a total volume of 180 ml, and centrifuged in 50 ml tubes for 10 min at 2,000 g. The pelleted spheroplasts were resuspended in about 10 volumes (105 ml per 10-12 g initial cell wt) of 18% Ficoll buffer (18% Ficoll 400 w/v, 20 mM Na-PIPES, 0.5 mM $MgCl_2$, 1.0 mM PMSF, pH 6.5) by adding 20-25 ml to each tube, covering it with Parafilm, and mixing at high speed on a vortex mixer for 1 min. The samples were pooled, and the lysate homogenized with one stroke of a motorized Teflon pestle homogenizer (Vitro 30 ml rotated at 1200 rpm). The homogenate (25 ml) was then layered over 20 ml of Glycerol-Ficoll buffer (20% glycerol v/v, 7% Ficoll w/v, 20 mM Na-PIPES, 0.5 mM $MgCl_2$, 1.0 mM PMSF, pH 6.5) in each of four 50 ml tubes, and centrifuged at 40 min at 25,000 g. The supernate was carefully removed by aspiration. The pellet at this stage consisted primarily of nuclei entrapped by cell membrane. The nuclei were released by suspending each pellet in 20 ml of 18% Ficoll buffer and mixing as before for 3 min, followed by centrifugation for 15 min at 3,000 g. The supernate, which contained the nuclei, was decanted into another tube, and spun for 15 min at 25,000 g. The pelleted nuclei were washed once by adding 20 ml of 18% Ficoll buffer to each tube, mixing for 2 min, and repelleting as above.

Nuclei were converted to insoluble chromatin by suspending the pellets with a spatula into a combined volume of 10 ml of digestion buffer (5.0 mM Na-PIPES, 0.5 mM $MgCl_2$, 0.05 mM $CaCl_2$ or 0.05 mM $SrCl_2$, 1.0 mM PMSF, pH 6.5), mixing on the vortex mixer until homogeneous, and incubating with agitation for 10 min at 37°. After a further mixing for 1 min, the suspension was centrifuged for 15 min at 12,000 g, and the supernate removed. This cycle was repeated two more times to produce the washed, insoluble chromatin pellet. The final yield was about 2.4-2.8

mg DNA as chromatin.

Nuclease digestion of yeast chromatin.

Insoluble chromatin was mixed into 9 ml of digestion buffer until homogeneous. Digestions were at 37° with about 400 U/ml micrococcal nuclease (Worthington Biochemical Corp.), and 300 µg/ml DNA as chromatin. The DNA concentrations were measured as in Lohr et al. (1), or by a modified DNA specific fluorimetric assay (19). Samples were withdrawn at designated times, and the reaction terminated by the addition, on ice, of 1/10 volume of 10 mM EDTA, 5 mM Na-PIPES, pH 6.5. After at least 15 min on ice, the samples were centrifuged for 10 min at 4° in a Beckman microfuge. The supernate was removed and divided into two aliquots, one of which was added to 0.5 volume of 3X native nucleoprotein sample buffer (see "Gel electrophoresis"). All samples were stored at -20°.

Ca^{++} induces both DNase and RNase activity with micrococcal nuclease; Sr^{++} is reported to induce only DNase activity (20). Since yeast nuclei have large amounts of RNA (21), we sometimes used Sr^{++} as the nuclease activator in our digestions. The results with either nuclease activator were qualitatively and quantitatively similar.

In some experiments, a small portion of the insoluble chromatin was digested with a mixture of RNase T1 and bovine pancreatic RNase (0.04 Sankyo units/ml and 0.15 Kunitz units/ml; both Calbiochem-Behring Corp.). Digestion and handling were as above.

Isolation and digestion of erythrocyte nuclei.

Frozen chicken erythrocytes were lysed in 10 mM Tris-HCl, 150 mM NaCl, 0.5 mM $MgCl_2$, 1.0 mM PMSF, pH 7.5, and the nuclei washed four times in this buffer (22). The nuclei were then washed three times by mixing on the vortex mixer in 18% Ficoll buffer, glycerol-Ficoll buffer, and again 18% Ficoll buffer, with centrifugations for 15 min at 25,000 g. This was done to mimic the treatment of yeast nuclei. Finally, the nuclei were subjected to two incubation washes in digestion buffer, and then digested and further handled exactly as with yeast.

Gel electrophoresis and analysis.

A modified high resolution native nucleoprotein gel was devised, based on the discontinuous buffer system of Williams and Reisfeld (23), and incorporating 30% glycerol as a nucleoprotein stabilizing agent (24). This gel system gives the same pattern of nucleoproteins in the mono- and dinucleosome regions as does the continuous system of Todd and Garrard

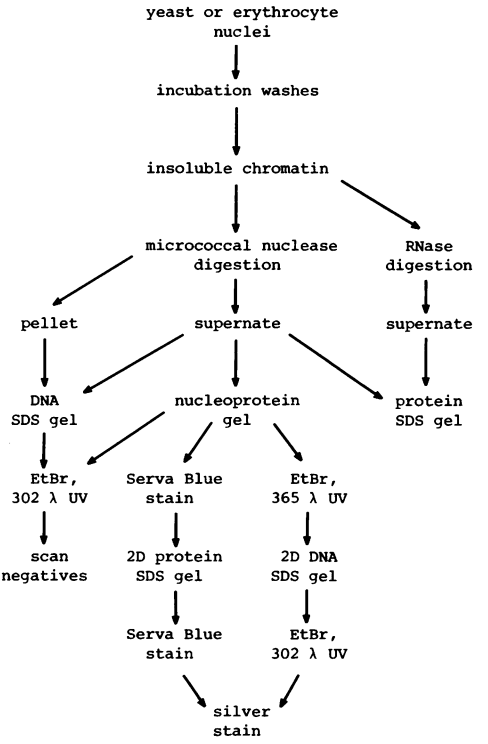

FIGURE 1. Flowchart of experimental procedure.

(24), but our gel resolves these species more sharply (25). The stacking gel consisted of 0.70% agarose in a buffer of 30% glycerol v/v, 0.5 mM EDTA (free acid), 15 mM Tris-H_3PO_4, pH 5.5. The separating gel was 6% acrylamide, 20:1 acrylamide:Bis, and contained 30% glycerol v/v, 0.5 mM EDTA, 15 mM Tris-HCl, pH 7.5. Running buffer was 0.5 mM EDTA, 15 mM 5,5'-diethylbarbituric acid (Sigma), adjusted to pH 7.0 with Tris base. Sample buffer (3X) was 90% glycerol v/v, 0.01% xylene cyanole FF (Bio-Rad), 1.5 mM EDTA, 45 mM Tris H_3PO_4, pH 5.5. Gels 0.8 mM thick and 13 cm long (separating gel, 8 cm long) were run at 75 volts and 4° for about 40 hr on a slab apparatus. The running buffer was recirculated. Full details on the pouring of these gels will be published elsewhere.

Other electrophoretic protocols, and the subsequent staining and quantitation of gels, are detailed in the figure legends. An outline of the analytic strategy is presented as a flowchart (Figure 1).

RESULTS

Experimental Rationale

Our experimental aim is to prepare yeast chromatin that is insoluble and free of contaminating RNA and DNA, digest with micrococcal nuclease, and then analyze the material that becomes soluble. We also analyze the DNA found in the complex insoluble fraction.

It is important that the chromatin be minimally perturbed during purification, because the maintenance of a native chromatin fiber could determine the excision pattern upon micrococcal nuclease digestion. Our chromatin isolation therefore incorporates at all steps a concentration of Mg^{++} (0.5 mM), which is sufficient to maintain the condensation of the 250 Å chromatin fiber in mammals (26-29). We also avoid exposing the preparations to chelating agents, detergents, and polyamines.

Prior to the isolation of nuclei, yeast cells are usually converted to spheroplasts with commercially available enzymes, which show significant amounts of DNase and protease activity (15). We therefore use Lyticase, which is an enzyme that in comparison is 500- and 10-40 fold reduced in DNase and protease activities, respectively (15). After osmotically lysing the spheroplasts, the lysate is centrifuged on a modified step gradient, so as to rapidly and cleanly separate crude nuclei from the cytoplasmic fraction, which contains high protease activities.

We employ a yeast strain that is free of mitochondrial DNA in order to avoid contamination arising from the co-isolation of this organelle with nuclei, since mitochondrial DNA accounts for about 20% of total DNA in wild-type yeast (30). Since yeast nuclei contain large amounts of RNA (21), soluble RNA and other nucleoplasmic components are released by pre-digestion washes of isolated nuclei that mechanically disrupt the nuclear membrane. The washes also quantitatively remove the ribosomes.

No detectable inner histone or acid precipitable DNA is released by this procedure, although a small amount of acid soluble DNA, equivalent to 4-7% of the total DNA, is released in the first wash. Furthermore, a control incubation at 37° for 3 hrs without nuclease releases no detectable protein or DNA. No evidence of proteolysis can be seen on SDS gels for proteins that have been solubilized by micrococcal nuclease.

We also prepare chicken erythrocyte chromatin by a method mimicking that employed for yeast. Soluble erythrocyte nucleoproteins are analyzed by native electrophoresis, in order to directly compare yeast to a well characterized complex eukaryote.

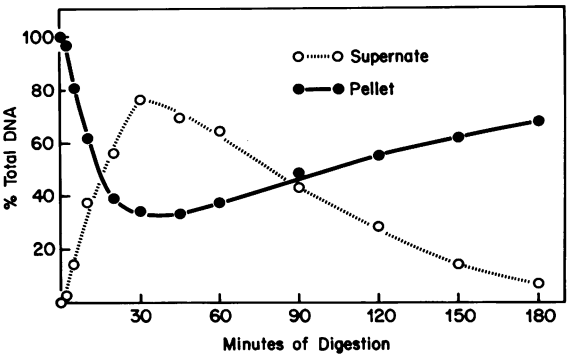

FIGURE 2. General distribution of DNA during digestion of yeast chromatin by micrococcal nuclease. DNA was quantitated by a DNA-specific fluorescent assay as in (19), except that the buffer was 50 mM Tris-HCl, pH 7.5, and 0.01% SDS and 100 mM $MgCl_2$ were included. The $MgCl_2$ stabilizes the fluorescence in the presence of SDS. The assay detects only acid precipitable DNA (19).

Pattern of DNA cutting by micrococcal nuclease.

Yeast chromatin can be separated into major insoluble and soluble fractions after digestion with micrococcal nuclease, as determined by a DNA specific assay (Fig. 2). Electrophoresis of purified RNase-treated DNA from these two fractions reveals strikingly different patterns of nuclease cutting (Fig. 3A).

At early points in digestion, a continuous distribution of DNA with no nucleosomal repeat is seen in the pellet. DNA of sizes less than about 150 base pairs (bp) appears to be relatively rare, although some such material is present. Larger DNA within this fraction is progressively lost with increasing digestion, but some nuclease resistant DNA persists as a background distribution throughout digestion (scans not shown). The persistent DNA has a continuous size distribution, mostly 150-1000 bp, that approximates the range of sizes found among the soluble, discrete oligonucleosomal series (see below). The amount of insoluble DNA reaches a minimum at a time (30-40 min) when soluble DNA is at a maximum; at this point about 30% of the total DNA is in the insoluble fraction. The material on the gel is DNA because quantitation by the scanning of gels agrees well with that obtained by the DNA-specific fluorimetric assay (data not shown).

At later stages of digestion, a short multimeric series of DNA bands of a normal yeast repeat size (160-165 bp, refs. 1,2) is observed in the

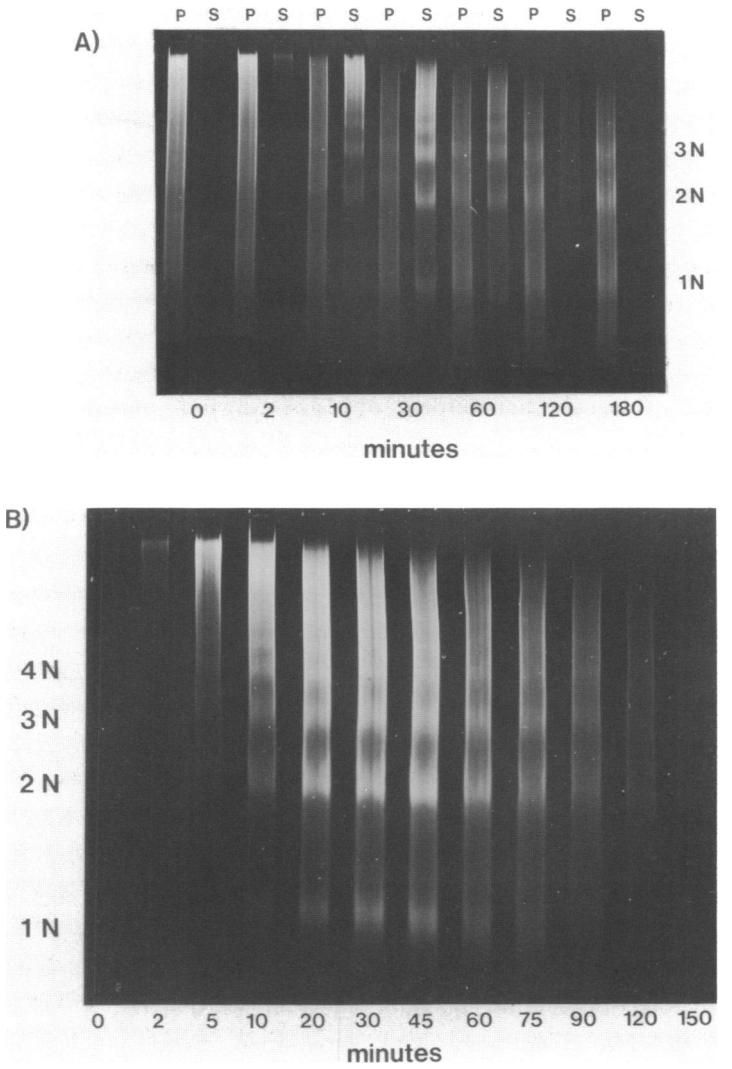

FIGURE 3. Gels of DNA from yeast chromatin digested with micrococcal nuclease. Equal volumes of samples from a Ca^{++} activated digestion were resolved on a 6% acrylamide gel (20:1 acrylamide: bis) using an SDS buffer system (35), and then stained with ethidium bromide. A) The distribution of DNA between soluble and insoluble chromatin (S, supernate; P, pellet) as a function of digestion. The pellets were treated with RNase (1) and pronase, then made 1% in SDS and phenol extracted, and finally treated with RNase a second time. Processed samples were diluted to the volume of the original digestion sample. The supernates were simply treated with RNase. B) The DNA patterns of the soluble fraction in detail.

pellet (Figure 3A). Approximately equal amounts of mono- and dinucleosomal DNA, and lesser amounts of tri- and tetranucleosomal DNA, are superimposed upon the background DNA distribution. The relative quantities of these DNA size classes vary little with the extent of digestion (scans not shown). The proportion of total DNA represented by these bands appears to be about 20-30%, if judged by the increase in pellet DNA from about 30-40 minutes of digestion onward (Fig. 2). The amount of DNA found in these bands increases significantly between 60 and 180 minutes of digestion, concomitant with the disappearance of most of the soluble oligonucleosomes. It therefore appears that these DNA bands derive from precipitation late in digestion. More exacting studies are needed to determine if these bands represent nucleosomes that are physically distinct from the bulk of the soluble nucleosomes. The precipitated material may account for the reported loss of about 20% of total yeast DNA upon extensive micrococcal nuclease digestion of nuclei (1).

DNA in the soluble fraction initially appears as high molecular weight material. At intermediate times of digestion, one observes a multimeric series of DNA bands that is resolvable to about the hexamer. Soluble DNA at the time of maximum solubility accounts for about 70% of the DNA at this timepoint (Figure 2).

Examination of the kinetic patterns of digestion for this oligonucleosomal DNA series reveals two unusual features (Figure 3B).

[1]. The DNA of the dinucleosome, trinucleosome, and tetranucleosome are present in a nearly constant ratio relative to one another throughout digestion, even when soluble DNA has virtually disappeared. Micrococcal nuclease is apparently attacking internucleosomal DNA in a selective, non-random manner. It is clear that a majority of these oligonucleosomes are processed by micrococcal nuclease into DNA species of subnucleosomal size or smaller, because there is no corresponding quantitative increase in precipitated mono- or oligonucleosomal DNA.

[2]. Mononucleosomal DNA is always present in markedly smaller amounts than any of the other short oligonucleosomal DNA size classes. If intact mononucleosomes are excised from this oligonucleosomal structure, most of them are apparently further digested at an increased rate to subnucleosomal or acid soluble DNA. Only a small proportion (about 10%) of all nucleosomes are found in the pellet as mononucleosomes; this amount is insufficient to account for the observed depletion in soluble

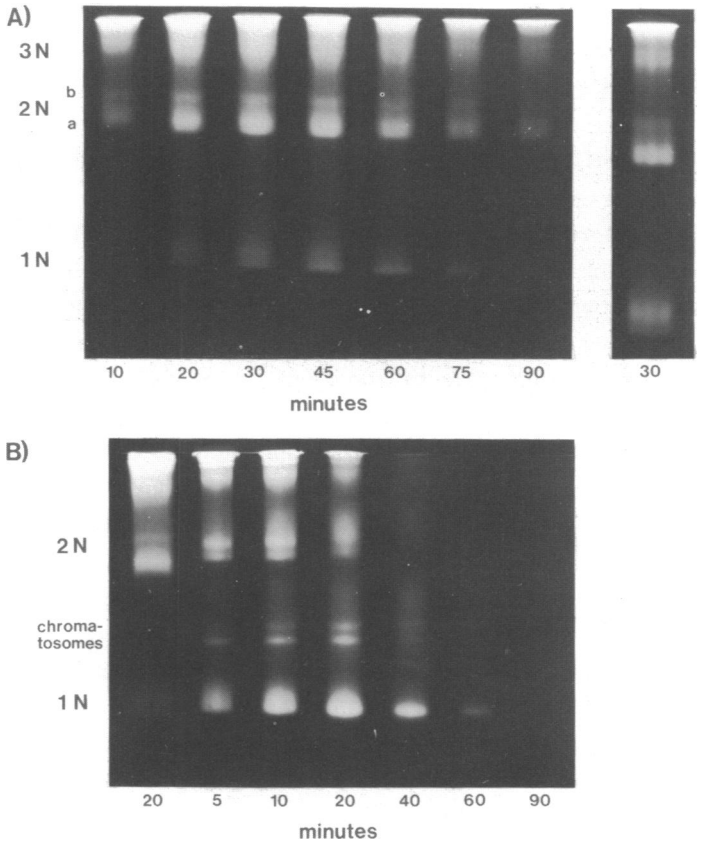

FIGURE 4. Native gels of nucleoproteins from yeast and erythrocyte (see Materials and Methods). Gels were stained with ethidium bromide. A) Tracks 1-7 are from a Ca^{++} activated yeast digest (see Figure 3), with sampling times as indicated. Track 8 is a 30 minute sample from a Sr^{++} activated micrococcal digest. B) Tracks 2-7 show nucleoproteins released from erythrocyte chromatin by Ca^{++} activated digestion. Track 1 is a coelectrophoresed sample from the above yeast experiment.

mononucleosomes.

Electrophoretic patterns of soluble nucleoproteins.

A time course of released yeast nucleoprotein (Figure 4A) reveals a simple, persistent pattern of two closely resolved trinucleosomes, two well resolved dinucleosomes, and two mononucleosomes, one of which is visible only upon silver staining (see below). We see no band which stains with ethidium bromide in the position where chromatosomes (nucleosomes containing HI or H5, ref. 31) migrate (Figure 4B). The leading yeast

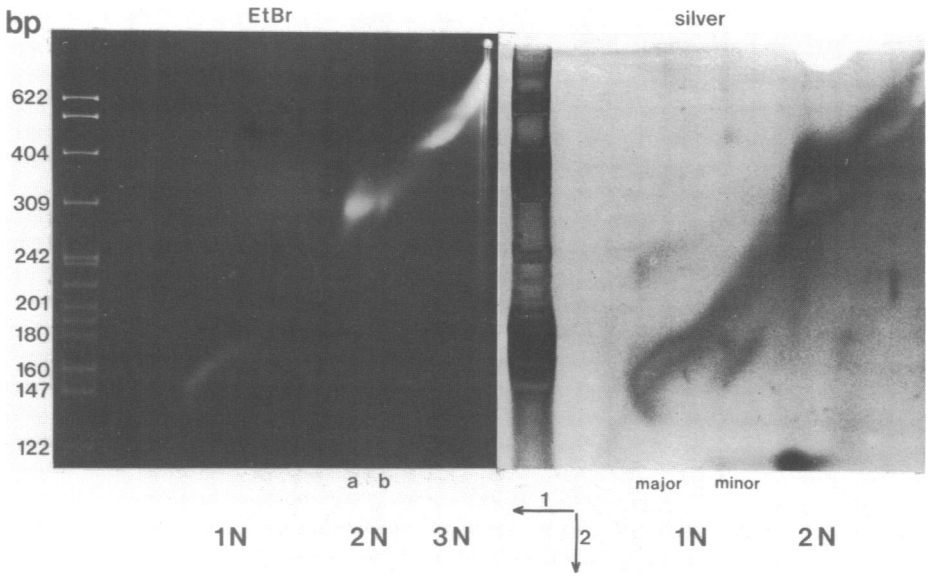

FIGURE 5. Two dimension gel of DNA from yeast nucleosomes. A nucleoprotein track from a Ca^{++} activated digest (see 45 minute timepoint in Figure 4) was visualized with ethidium bromide, and then run in the second dimension on an SDS gel system (see Figure 3). The gel was sequentially stained with ethidium bromide and silver (37). pBR322 plasmid was digested with the restriction enzyme Hpa II to provide DNA size markers.

dinucleosome has a higher mobility than the leading erythrocyte dinucleosome, whereas the trailing yeast dinucleosome is slightly slower than its erythrocyte counterpart. As digestion proceeds, the two dinucleosomes are present in a constant ratio of approximately 4:1 (scans not shown). The amount of dinucleosomal material exceeds the amount of mononucleosome throughout digestion, in agreement with the results of DNA gels.

We find two erythrocyte chromatosomes in addition to the core nucleosome, as has been reported (32). Unlike yeast, the erythrocyte core particle accumulates continuously relative to larger soluble nucleoproteins during the course of digestion (Figure 4B).

DNA components of soluble nucleoproteins.

To analyze the DNA components of electrophoretically resolved yeast nucleoproteins, second dimension gel electrophoresis was performed in the presence of SDS (Figure 5). Upon silver staining, a minor DNA band

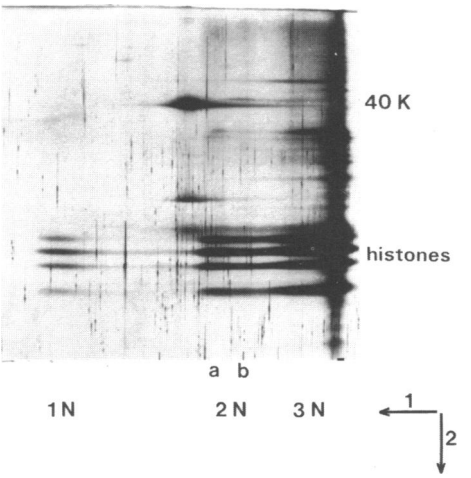

FIGURE 6. Two dimension gel of proteins from yeast nucleosomes. Track from a native nucleoprotein gel was stained with Serva Blue (Serva Fine Biochemicals), equilibrated with stacking buffer, and polymerized onto a 6% acrylamide stacking gel. The separating gel was 18% acrylamide (37.5:1). The SDS buffer system was that of Laemmli (33), except that 0.01% mercaptoacetic acid was freshly added to the running buffer as an electrophoresable anionic reducing agent (36). The 40,000 dalton protein is always very prominent, running ahead of the two dinucleosomes, a and b, in the native gel.

corresponding to a second mononucleosome is revealed.

The DNA length of the minor component is similar to that of the major mononucleosome. The lower size limit of mononucleosomal DNA is about 146 bp, but this core size DNA never appreciably accumulates. The DNA of the major species gradates into a roughly parallel DNA streak of diminishing intensity, giving the region an overall zig-zag appearance. It is noteworthy that the DNA lengths of this mononucleosome tail (170-270 bp) greatly exceed the reported average yeast repeat length of 160-165 bp (1,2). The average DNA size of the two dinucleosomes is roughly the same, centering on about 330 bp, although the major species has a broader range. These data are consistent with previous work (1,2).

Protein components of soluble nucleoproteins.

Analysis of the protein components of yeast nucleoproteins was also performed by second dimension SDS gel electrophoresis. Yeast mono- and dinucleosomes possess a similar distribution of core histones (Figure 6).

We note that silver staining is not linear, but that the staining pattern of the histones follows that of the Serva Blue stained histones of

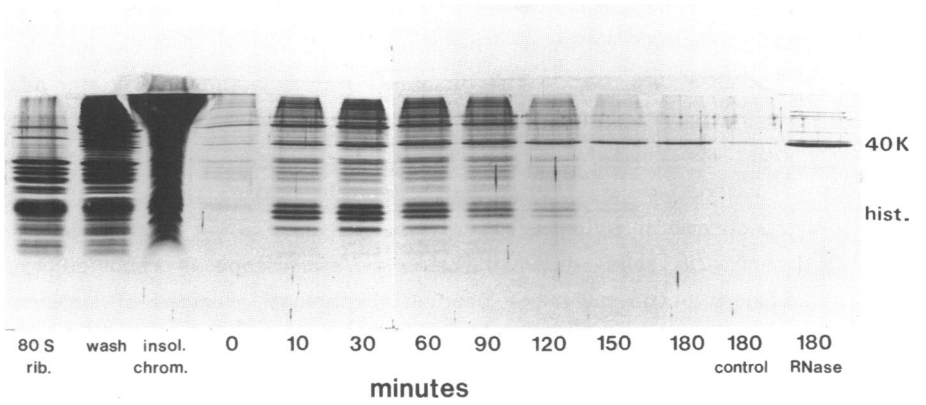

FIGURE 7. SDS gel of total yeast proteins solubilized by nuclease. Equal volumes of supernate from each sample point were loaded directly onto an 18% acrylamide gel (see Figure 6). Gel was stained with Serva Blue. Most of the minor low molecular weight peptides released by micrococcal nuclease comigrate with bands from an 80S yeast ribosome preparation (track 1, ref. 34). The washes remove almost all of the ribosomal proteins, as well as additional high molecular weight species (compare track 2 with tracks 5-8).

the total soluble chromatin (Figure 7). No other prominent bands are seen in positions corresponding to the yeast mono- and dinucleosomes.

Erythrocyte chromatosomes contain H5 in the leading nucleoprotein band, and the two HI subtypes in the trailing component, as previously reported (31,32) (data not shown).

Non-histone proteins solubilized by nuclease

Prior to the digestion of the insoluble chromatin preparation with nuclease, the incubation washes remove a large number of proteins, including bands that comigrate with a yeast 80 S ribosome preparation (Figure 7).

After digestion to various extents by micrococcal nuclease, a complex and highly reproducible complement of proteins is observed in the soluble fraction. The overall presence of the non-histone proteins closely correlates with the solubilization and subsequent precipitation of the core histones as a function of nuclease digestion, with the single exception of a very prominent band of 40,000 daltons (molecular weight determined by SDS gel electrophoresis). When insoluble chromatin is treated by RNase alone, only this 40 kilodalton protein and four minor higher molecular weight peptides are solubilized (Fig. 7).

DISCUSSION

A large proportion of yeast chromatin could not be solubilized by micrococcal nuclease digestion. This insoluble material appears initially as a continuous distribution on a DNA gel. Such a DNA pattern has previously been observed for yeast very early in digestion (1), but no chromatin fractionation was employed, and therefore there was no information on chromatin solubility.

Yeast chromatin seen under the electron microscope is reported to have, in addition to the prevalent beaded morphology, regions of smooth fiber, containing DNA apparently associated with protein, and sometimes nascent ribonucleoprotein fibrils (38). Smooth chromatin regions have been correlated with high transcriptional activity (39,40). These smooth fibers perhaps do not contain nucleosomes, and may give rise upon digestion to the continuous DNA pattern observed in our experiments. They may also account for the significant amounts of interband DNA that have been observed for unfractionated micrococcal digests of yeast nuclei (1,3).

A similar continuous DNA pattern is also observed in complex eukaryotes, when the DNA from mild micrococcal digests of chromatin is probed with genes known to have high rates of transcription (41-44). Recently, it has been found that after micrococcal nuclease digestion and fractionation by solubility, 90% of the mouse ribosomal RNA chromatin in an actively expressing cell line is insoluble and gives a continuous gel pattern (45). In mouse liver that is weakly expressing, most of the rRNA chromatin is soluble and gives a nucleosomal DNA repeat (45). A similar phenomenology has been reported for the C_k Ig L chain gene in two murine cell lines that respectively express or do not express this cistron (46).

Taken together, the above observations suggest that the bulk insoluble DNA smear that we see in yeast may represent actively transcribing chromatin. Proof of this, of course, requires studies of yeast chromatin using probes from active genes.

It may be asked why we observe such a major insoluble, non-nucleosomal fraction that has hitherto gone unreported in yeast. Although we cannot be certain, this may be due to the fact that we have fractionated the nuclease digests, while others have not. In this connection, we note that a fraction of mammalian chromatin, 5-15% of the total, also cannot be solubilized by digestion with micrococcal nuclease

(45,46).

A possible explanation for insoluble chromatin is that transcribing or immediately transcribable chromatin is associated in insoluble form with the nuclear matrix, an insoluble structure which in complex eukaryotes is reported to be selectively associated with highly transcribed genes (47,48) and RNA processing (49). If this is the case with yeast, then the amount of insoluble chromatin may be in flux, its association with the matrix being dependent on the transcriptional state of the cell and the methods by which the chromatin is isolated. At least 40% of the yeast genome is transcribed into polyadenylated RNA (50), more than enough to account for the amount of insoluble chromatin that we observe.

The non-random excision pattern manifested by the constant ratio of soluble short oligonucleosomes is unusual. It is consistent with a set of closely packaged short polynucleosomal structures (but not necessarily higher order), each of which is demarcated at the ends by nuclease accessible features. Nuclease access to spacer DNA within these structures is severely restricted, so that processing to smaller sizes occurs primarily by the elimination of mononucleosomes from the ends. We have no evidence that processing by the nuclease normally involves the excision of intact mononucleosomes from these polynucleosomes (see Figure 8).

Our results differ from those of previous studies of yeast, in which it was found that mononucleosomal DNA accumulates rapidly at the expense of larger fragments during digestion (1,2), a pattern consistent with random nuclease cutting at linker DNA (1). A possible explanation for these apparently contradictory results is offered by the observation that Miller spreads of yeast chromatin reveal heterogeneous spacer DNA lengths greatly in excess of that expected on the basis of a 165 bp micrococcal nuclease repeat, whereas similarly prepared and co-spread mouse chromatin showed a more typical, relatively invariant linker length (38). Rattner et al. suggest that, in yeast, the core DNA may be especially labile to peeling from the histone octamer under certain experimental conditions (38). If so, the linkers would be artifactually exposed to nuclease action, allowing easy cleavage between nucleosomes in a nearly random manner.

DNase I studies have demonstrated that much, and possibly all, of yeast chromatin has a spacer length that is phased at 10n + 5 base pairs, a feature not found in erythrocytes or HeLa (7). From this information, it has been hypothesized that yeast nucleosomes pack edge-to-edge in the

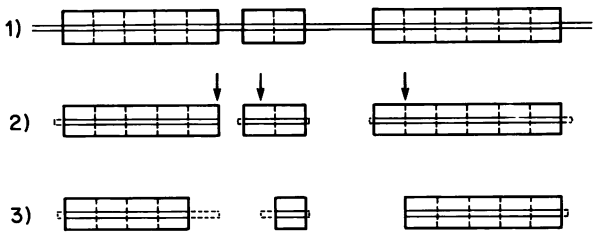

FIGURE 8. Model for the processing of polynucleosomes by micrococcal nuclease. Short polynucleosomal arrays of various sizes are initially cleaved out of chromatin at relatively nuclease sensitive features (═). The schematic representation of these arrays is not meant to reflect the unknown in vivo geometry of packing. Nucleosomes at the ends of arrays are then eliminated with roughly equal probability by exo- or endonucleolytic cleavage at sites within the core nucleosome (↓). Intracore cleavage of a dinucleosome would generate the observed mononucleosome tail (Figure 5). Although many other models for processing are possible, this simple scheme is attractive because it accounts for the unusual digestion kinetics and the depletion of the mononucleosome as a coordinate phenomenon.

chromatin fiber in a structure with a dinucleosomal repeat (7,51). One explanation of the relative resistance of yeast oligonucleosomes as small as the dinucleosome to our digestions may be that the linker region is arranged differently (or the nucleosomes are interacting differently) than in the bulk chromatin of most eukaryotes, where nucleosomes may pack in a different way (29,51,52). We find that digestions of erythrocyte nuclei, under the same conditions as yeast, show no such dinucleosomal nuclease resistance among soluble nucleoproteins.

Supportive evidence for a different linker arrangement in yeast may be provided by the mononucleosome tail. This tail, which contains DNA sizes much larger than expected on the basis of a 165 bp repeat, may be formed by asymmetric nuclease cleavage of dinucleosomes at a site outside of the linker.

The lack of a chromatosome in yeast further suggests that its mode of chromatin organization differs strongly from that of the erythrocyte. The chromatosome, which consists of the 146 bp core nucleosome, about 20 additional bp of DNA, and one molecule of H1 or H5 (31), has been implicated as the basic repeating structural unit of the metazoan 250 A chromatin fiber (27,51,53). H1 has not yet been found in yeast (2,8,12). It has been proposed that the core histone octamer is capable of organizing 168 bp of DNA in the absence of H1 (54). If this is true, it would be

consistent with a reduced accessibility to nuclease of the DNA between adjacent core nucleosomes in yeast.

Soluble yeast mononucleosomes do not appear to have a pronounced resistance to further digestion at the core particle DNA size (146 bp), as do those from erythrocyte. A simple interpretation of this phenomenon is that the DNA-histone interactions of the excised yeast mononucleosomes are atypically weak, particularly at the ends of the core DNA. Evidence for this view is offered by the above-cited microscopic study (38), and also by the report that is has so far been impossible to prepare stable mononucleosomes with DNA sizes narrowly distributed around 146 bp (13). It is also possible that isolated mononucleosomes have altered histone-histone interactions, which make specific DNA sites within the core more susceptible to the nuclease. Yeast histones do not reconstitute into nucleosomes by techniques successful with chicken or calf histones (13); yeast H3 interacts in solution without the formation of α-helix secondary structure, unlike H3 from calf or pea (8). Yeast nucleosomes are apparently stabilized (rendered resistant to micrococcal nuclease) when associated with at least one other nucleosome.

A striking feature of the solubilized nucleoproteins is that they are electrophoretically resolved into two bands for each DNA multimer of the nucleosomal repeat, from the monomer to at least the trimer. This dualism is most easily examined experimentally for the case of the dinucleosomes. Electrophoresis in a second dimension reveals no stoichiometric non-core proteins that might readily account for the difference in mobility . The two mononucleosomes and the two dinucleosomes are respectively well-separated on the native gel, perhaps indicating significant physical differences between members of a doublet. The basis for the resolution of these bands is not yet understood, and analogous distributions in other eukaryotes have not been reported. Despite the fact that mono-, di-, and trinucleosomes all show doublets, it does not necessarily follow that the soluble fraction of the chromatin contains two functional classes.

The 40 kilodalton yeast protein is interesting because it is present in a large amount in our soluble chromatin preparation. The selective solubilization of this protein by RNase is consistent with its being a component of a heterogeneous ribonucleoprotein (hnRNP), but more data is needed for any identification. HnRNA has been reported to be associated with chromatin (55). We do not know whether this protein is associated in vivo with oligonucleosomes, nor whether it may be related in some fashion

to the unusual digestion pattern that we observe.

The 5' region of the chicken ovalbumin gene, when blot-hybridized to the DNA of hen oviduct nuclei that have been digested to various extents with micrococcal nuclease, gives a pattern of relatively nuclease resistant DNA bands of about 500 and 300 bp (44). The sizes of these bands are smaller than for the corresponding bands of bulk chromatin. Little or no mononucleosomal size DNA is observed (but this DNA size class may hybridize inefficiently) for this active gene. In mouse liver, soluble rRNA chromatin gives a nucleosomal repeat of 168 bp, which is significantly shorter than the bulk repeat of 190 bp (45). Again, no mononucleosomal DNA is seen, but control experiments rule out hybridization or blot-transfer artifacts for this case (45). The bulk properties of the yeast soluble fraction resemble these reports in terms of repeat size and the depletion of the mononucleosome. These parallels suggest that the general structure of soluble yeast chromatin may correspond to a chromatin configuration found in metazoan genes that have the potential to be transcribed at high rates.

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