The Salt Dependence of Chicken and Yeast Chromatin Structure

EFFECTS ON INTERNUCLEOSOMAL ORGANIZATION AND RELATION TO ACTIVE CHROMATIN*

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The ionic strength dependences of yeast and chicken erythrocyte chromatin structure have been examined by analysis of nuclear DNase I and Staphylococcal nuclease digestions done under various salt and divalent cation concentrations. The basic features of yeast DNase I profiles (intracore/intercore patterns and their 5-base pair offset) remain present under all conditions tested. However, there are changes in specific parts of the patterns. In very low salt, the intercore DNase I pattern is enhanced; even very small intercore bands can be detected. Staphylococcal nuclease intracore cleavage becomes prominent. Increasing salt enhances the large DNase I intracore bands and the frequency of spacer cleavage for both nucleases. Thus, yeast has a salt-dependent higher order structure: a chromatin fiber with a prominent spacer/core distinction in (physiological) salt; a fiber with a decreased distinction between spacer and core, i.e. a more uniform fiber, in very low salt. The salt-dependent bulk changes resemble single gene chromatin changes during gene expression and may provide a model for that process.

Above bands 16.5–17.5, chicken and yeast intercore patterns are coincident. Thus, at least a fraction of chicken chromatin has discrete length spacers like yeast does. This fraction may be significant, for the prominence of the intercore pattern, and hence the apparent abundance of discrete spacers, can be significantly enhanced by digestion in very low salt. The major differences between the two chromatins are in the intracore/intercore transition region; the region is larger and more complex in chicken; ionic strength changes affect the chicken transition region more strongly. Since this region of the profile corresponds to digestion near the ends of the core, that part of the nucleosome must differ in structure and in conformational flexibility in the two chromatins.

One technique which has yielded some information about spacer DNA organization is nuclearase (McGhee and Felsenfeld, 1980a) or chemical (Cartwright et al., 1983) cleavage of nuclear chromatin. For such studies, it is best to use cleavage agents which do not preferentially destroy the spacer. Of the agents commonly used, DNase I does this most successfully (Lohr et al., 1977a; Lehr and Van Holde, 1979).

A major component of the DNA-histone interaction is electrostatic and thus ionic strength-dependent. I have used this dependence, combined with the ability of DNase I to yield information on spacer organization, to probe the higher order structure of yeast and chicken erythrocyte chromatin. Such an analysis is particularly important for yeast. Previous nuclease digestion data have suggested a zigzag arrangement of nucleosomes in yeast chromatin (Lohr and Van Holde, 1979). However, these data were all gathered at low ionic strength and Thoma et al. (1979) showed that in rat liver chromatin such a zigzag structure was present only at low ionic strength. Results presented here show that most of the features in the yeast low salt DNase I patterns remain present under other conditions and thus reflect permanent features of yeast chromatin. However, there are some ionic strength-dependent changes which suggest new information about spacer DNA organization and higher order chromatin structure in yeast.

Yeast chromatin differs from most eukaryotic chromatin in several ways: small nucleosome repeat length; no known H1 histone; no observable chromosome condensation; transcription of a significant (~40%) portion of the genome in growing cells (Hereford and Rosbash, 1977). It is therefore important to compare yeast chromatin results to results from other chromatins. Chicken erythrocyte is a well studied chromatin (McGhee and Felsenfeld, 1980a) which appears to be fairly typical in terms of higher order structure and response to ionic strength changes. It provides an especially interesting comparison for yeast because the two represent a very striking contrast in genomic level of transcriptional activity. Thus, I also analyzed the response of chicken chromatin structure to ionic strength changes, using nuclease digestion as a probe.

Studies of both chromatins depend on analysis of DNase I digestion products. Staphylococcal nuclease results are used for confirmation. The effect of ionic strength on staphylococcal nuclease (Weischet et al., 1979) and endogenous nuclease (Vanderbilt et al., 1982) digestion patterns from metazoan chromatin have been analyzed previously. DNase I analysis is especially useful because it yields information about spacer organization and because the enzyme is relatively free of nuclease-associated artifacts such as exonucleases and high levels of sequence-specific cleavage. Analysis of nuclease cleavage products is useful because, in studies such as these, one wants to minimize changes in nucleosome properties. Ionic strength should have less effect on the mode of nuclease cleavage than it does on the rate of cleavage. Rates of cleavage,
chromatin or naked DNA, can be strongly affected by the ionic strength (Weischt et al., 1979).

MATERIALS AND METHODS

Nuclease Digestion—Yeast nuclei were isolated as described previously (Lohr, 1981). Nuclease digestions under all the various conditions were always performed on aliquots from the same nuclear preparation to avoid the possibility of variations between nuclear preparations. Nuclei were resuspended in the standard yeast digestion solution: 1 M sorbitol, pH 6.5, 0.06 mM Ca\(^{2+}\) for staphylococcal nuclease; the above plus 0.5 mM Mg\(^{2+}\) for DNase I. To various aliquots of these nuclei, salt (30–350 mM NaCl) or cations (3 mM Ca\(^{2+}\) or Mg\(^{2+}\) or 0.15 mM spermine, 0.6 mM spermidine, cf. Hewish and Burgoyne, 1975) were added, with mixing. For the very low ionic strength digestions, aliquots of pelleted nuclei were washed in the low ionic strength digestion buffer (1 M sorbitol, pH 6.5, 6.05 or 0.01 mM Mg\(^{2+}\), 0.005 mM Ca\(^{2+}\)), repelleted, and then resuspended in the same buffer. There was no evidence of nuclear lysis under these conditions. It is not possible to know the true concentration of divalent cations in the very low salt nuclei because of the possibility of specific binding of these cations to chromatin. However, the ionic strength is clearly much lower after washing and subsequent resuspension under these conditions, and there are clearly pattern differences (see text).

Chicken erythrocyte nuclei were isolated by a modification of the technique described by Shaw et al. (1976) using reticulocyte standard buffer instead of 0.15 M NaCl to stabilize the nuclei. After isolation, the nuclei were resuspended in Tris (10 mM), sucrose (0.3 M), pH 7.2, and salt and/or divalent cations were added as described above. For very low salt digestes, the nuclei were washed and then resuspended in 0.3 M sucrose at the appropriate divalent cation concentration. In contrast to yeast nuclei, chicken nuclei frequently lyse in this ionic strength buffer. Chicken nuclei were also resuspended and digested in yeast digestion solution, 1 M sorbitol, pH 6.5, 0.05 mM Ca\(^{2+}\), 0.5 mM Mg\(^{2+}\). Digestion patterns looked like typical (0.5 mM Mg\(^{2+}\)) chicken patterns. In all cases, the nuclei were equilibrated for 5 min on ice. The nuclei were then placed at the required temperature: 30 °C for most digestions; 10 or 12 °C for low temperature digestes. Nuclease was added at various concentrations, depending on the digestion conditions, aliquots were removed at various times, adjusted to 10 mM in Na/EDTA, and cooled on ice. DNA was extracted as described previously (Lohr et al., 1977b): For the low temperature digestions, it is necessary to increase significantly the amount of nuclease used, particularly for staphylococcal nuclease digestes of yeast. We could not get significant levels of staphylococcal nuclease digestion at 4 °C without adding very large amounts of nuclease or waiting unacceptable times. This is not totally surprising given the relative insensitivity of yeast to staphylococcal nuclease. Digestions under other conditions could be carried out with, at most, modest increases in nuclease concentrations.

Electrophoresis—DNA from all nuclease digestes was electrophoresed on denaturing 5% polyacrylamide, 4:1 bisacrylamide, 7 M urea gels. The gels were stained in 0.5% of ethidium bromide and photographed on Polaroid Type 55 or Kodak Tri-X film under UV illumination. Negatives were scanned on a Joyce-Loebli microdensitometer.

Data Analysis—There is some digestion extent dependence of the relative intensities of bands in a DNase I pattern, particularly intracore bands. Therefore, in studies like these, compared samples should be digested to the same extent. For many of the samples in this work, DNA molecular weight distribution profiles on a gel were used to choose comparably digested samples. In the analysis of the 50 and 350 mM NaCl digestes, DNA size profiles strictly identical to profiles from lower salt digestes could not be obtained for brief extents of digestion. However, since the changes observed in this work are confined to only a few bands in the total pattern, the bands in the case of the profiles provide an internal standard to aid in choosing comparably digested 150 and 350 mM NaCl digest samples to be analyzed. In these cases, I also compared the salt digest profile to low salt profiles both more and less extensively digested. A band anomaly present in the salt digest had to be absent in both low salt samples to be considered. Acid soluble pH 3.0 is too imprecise to be used for comparison of these limited digests and various digestion conditions could affect the generation of acid-soluble nucleotides.

RESULTS

Bulk Yeast Chromatin

DNase I

DNase I digestion of yeast nuclear chromatin produces two regular series of 10.5-bp spaced bands: the intracore pattern, arising from nicks within the same core particle; the intercore pattern, arising from nicks in two neighboring core particles. Bands in these two series are identified by number. In digests under standard conditions (0.5 mM Mg\(^{2+}\)), the intracore pattern is always observed up to band 11 and to band 12 in moderate extents of digestion. The intercore pattern starts at a band called 11.5 and continues to larger sizes. Bands in the two series can be distinguished in the regions where they overlap because the intercore bands contain a 5-bp increment (Lohr and Van Holde, 1979). The 0.5 in their number, i.e. 11.5, denotes this. Both patterns are thought to arise from the same DNase I digestion sites within the core particle. A fragment falls in one or the other pattern depending simply on whether the next nick along the strand happens to be in the same or a neighboring core particle (Lohr and Van Holde, 1979). The size of a band in nucleotides is the average spacing (10.5 bp) times the band number (Lohr and Van Holde, 1979). There is also digestion within spacer DNA. This digestion appears to be random, involving no selectivity in the fraction of spacers which is cleaved or in the sites of cleavage. Spacer cleavage is probably the source of the background smear of DNA present in all DNase I profiles (Lohr and Van Holde, 1979).

Very Low Ionic Strength—The DNase I pattern produced by digestion under the ionic conditions used previously (0.05 mM Ca\(^{2+}\), 0.5 mM Mg\(^{2+}\)) is shown in Fig. 1A, lane 2. Although these are low salt conditions, the strong effect of divalent cations on chromatin makes this an intermediate concentration with respect to higher order condensation (Thoma et al., 1979). It is also above the concentration at which the major low salt core particle unfolding transition occurs in vitro (Gordon et al., 1978; Crothers et al., 1978).

Patterns from digestes performed in very low ionic strength (0.005–0.01 mM Mg\(^{2+}\), 0.05 mM Ca\(^{2+}\)) differ somewhat. Small intercore bands, mainly 5.5, 6.5, and 7.5, are produced with greatly increased frequency (Fig. 1A, densitometer traces 1 versus 2, 0). Their absence in track 2 is not a result of the greater intensity of the surrounding intracore bands in that track, for they remain absent in 0.5 mM Mg\(^{2+}\) profiles with lower intracore intensities and remain present in 0.005 mM Mg\(^{2+}\) digestes with higher intracore intensities in this region (not shown). These small intercore bands also remain detectable in extensive digestes (Fig. 1A, scan 5). The observation of intercore bands smaller than 11.5 confirms an important prediction of the intracore/intercore explanation for the two yeast DNase I ladder patterns (Lohr and Van Holde, 1979) which was never satisfactorily fulfilled in previous work.

The very low salt digestes also show better resolution of the larger (>11.5) intercore bands, particularly in the region around the nucleosome repeat length, i.e. 13.5–16.5 (Fig. 1A, lanes 1–3). This appears to result from a lower background of DNA in these profiles. Note, for example, the higher peak/interpeak ratio for these bands compared to the same bands in the 0.5 mM Mg\(^{2+}\)- or NaCl-containing digestes (Fig. 1A, scans 1–4). The background level is sensitive to digestion extent, being highest in brief digestes. That does not explain the differences between lanes 1 and 3, which are extremely

D. Lohr, unpublished observations.
FIG. 1. Effects of ionic strength changes on DNase I digestion patterns from yeast nuclear chromatin. All digestions were performed in aliquots of nuclei from the same preparation with various additions as described under "Materials and Methods." DNA was electrophoresed as described under "Materials and Methods." Bands in the yeast intracore pattern will be identified by — and/or by number; bands in the intercore pattern will be identified by $\bullet$. Electrophoresis is from top to bottom. Those scans which correspond to gel track photographs are numbered with the same number as the gel tracks. Scans for which there is no corresponding photograph will be.
closely matched in digestion extent, but it is the reason that the intercore bands are as clear as they are in scan 4.

This increased clarity in very low salt digests has an unexpected benefit. In previous work, the yeast intercore pattern could never be resolved beyond bands 24.5–25.5, ~260–270 bp (Lohr et al., 1977a), and thus we could not know whether the pattern extends to DNA sizes larger than two nucleosomes. This is an important question concerning the possible existence of a dinucleosome repeat in the structural organization of chromatin (Burgoyne and Skinner, 1981; Khachatrian et al., 1981). In very low salt digests, the background is low enough to see detail in these higher regions. Just above band 24.5 (or sometimes 25.5) there is a region in which the pattern loses resolution (Fig. 1B, scan LO). This blurred region resembles the intracore/intercore transition region between bands 11 and 12.5, as it appeared in some of the initial characterizations of yeast DNase I patterns (cf. Lohr et al., 1977a). The two regions are separated in size by one core particle length of DNA (24.5 – 11 = 13.5 x 10.5 = 147 nucleotides). Above the higher blurred region, just as above the lower (bands 11–12.5) one, a band pattern resumes (Fig. 1B, scan LO). At least 7–8 bands can be resolved in this highest pattern. The relative intensities of these bands show the same trends seen in the bands above the intracore/intercore transition region; the greatest intensity is in the first few bands after the transition, with intensity dropping off gradually as one moves to larger DNA sizes. The similarities to the intercore pattern, which is produced from digestion in neighboring nucleosomes, suggest that these largest bands reflect digestions within nucleosomes one removed from each other, i.e. separated by an intervening nucleosome. Thus, the intercore pattern and the structure giving rise to this pattern extend beyond a dinucleosome unit, at least in very low salt. This pattern may also be present under other conditions, but the higher background level obscures it. For example, a few bands remain visible in 0.5 mM Mg²⁺ digests (Fig. 1B, scan 0), but none can be detected in the 80 mM NaCl digest (Fig. 1B, scan 80).

Increasing Ionic Strength—With increasing NaCl concentration, parts of the intercore pattern become more prominent and parts of the intercore pattern become less prominent. These changes are again most reliably analyzed in densitometer scans. Bands 11.5 and 12 are particularly useful for analysis. In the standard (0.5 mM Mg²⁺) conditions, bands 11.5 and 12 are both clearly present in brief digest (Fig. 1A, scan 2), while band 11.5 dominates in more extensive digestes (not shown). In the very low salt digestes, band 12 is always a minor band (Fig. 1A, scan 1). For example, it is barely resolved in scan 1. In 80 or 150 mM NaCl, band 12 is always a major band (Fig. 1A, scans 3 and 4), even in extensive digestes (Fig. 1A, lane 6). On the other hand, band 11.5 is quite prominent in very low salt digestes (Fig. 1A, scan 1) but cannot even be detected in the 80 or 150 mM NaCl digest (cf. Fig. 1A, scan 4). The persistence and enhanced intensity of band 12 and the loss of band 11.5 in the salt-containing digestes demonstrate clearly that there are salt-dependent pattern changes.

Other bands in this same region are also affected (Fig. 1A, scans 1 and 2 versus 4); intracore bands 10 and 11 are enhanced; intercore band 12.5 is diminished. The concentration dependence of these salt-induced perturbations is shown in greater detail in Fig. 2, using data from a higher resolution gel. Up to 150 mM NaCl, bands 11 and 12 become progressively more prominent. Band 11.5 has disappeared by 80 mM NaCl and band 12.5 becomes very minor by 150 mM NaCl. Thus, increasing salt results in a progressive change which enhances the intracore pattern and diminishes the intercore pattern in the size ranges between bands 10 and 13.5. There is never an intracore band larger than band 12 under any conditions.

It is important to note that each set of compared samples in Figs. 1 and 2 were run on the same gel, so the differences observed cannot arise from gel to gel variations or staining or photographic artifacts. These analyses have been carried out in gels of varying composition, using different DNase I digestes, and the differences are all highly repeatable. Thus, they reflect a genuine salt-dependent perturbation of the DNase I digestion profiles of yeast chromatin.

Other parts of these patterns show little or no change with changes in ionic strength. For example, despite the large differences involving intracore bands 10–12, intracore bands 6–8 remain similar in intensity in 150 mM and lower salt digestes (Fig. 1A, scans 1 and 2 versus 4). Also, the intercore pattern remains, with bands of the same size, under all conditions (cf. Fig. 1B, scans LO, 0, and 80). Because most of the DNase I pattern is preserved, the basic elements of yeast chromatin structure must remain intact in the various conditions. The changes must reflect perturbations of this basic structure rather than large scale chromatin remodeling.

I also examined the effect of 350 mM NaCl. In brief digestes, there is a high background level and bands are very poorly

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**Fig. 2. High resolution analysis of the most salt-dependent region of the DNase I pattern.** Densitometer traces of gel tracks from a 0.5 mM Mg²⁺ (O) and several salt-containing digestes are shown. The NaCl concentration (in mM) is shown to the right of each trace. The dotted lines show the positions of bands 12 and 11.5. Intercore bands are identified by ●. Electrophoresis is from left to right.
resolved. More extensive digests produce a pattern which qualitatively resembles the 150 mM NaCl digest—enhanced bands 11 and 12, decreased bands 11.5 and 12.5 (Fig. 2, 350). There is also a slight shift in position of band 12.5 in these profiles; the peak lies halfway between the normal position of band 12.5 and the position of band 13, if it were detectable. No intracore bands above 12 can be resolved in these digests. Yeast nuclei are unstable in 350 mM NaCl and usually lyse. Thus, I have not studied the salt dependence further.

As discussed above, the higher level of background DNA in the intercore pattern from 150 mM NaCl digests suggests an enhanced frequency of spacer cleavage under these conditions. In agreement with this suggestion, one can also detect stronger peaks at nucleosome repeat length multiples in these profiles (Fig. 1B, scans 0 versus 150). In organisms with larger spacers and thus larger targets for spacer cleavage, such peaks are quite clearly seen (see below).

Since divalent cations have a particularly striking effect on chromatin structure (Thoma et al., 1979), I analyzed the effects of increased [Mg²⁺] and of the polyamines spermine/spermidine on yeast DNase I digestion patterns. These conditions produce only limited changes. At low extents of digestion, the normally resolvable bands 11.5 and 12 are replaced by a single peak lying between the normal positions of these two (Fig. 3, scans 2 versus 3, X). In moderate and extensive digests, the peak positions of intracore bands 10 and 11 are shifted to smaller sizes and the peaks are broadened, particularly on the low molecular weight side (Fig. 3, scan 4, X). Intracore bands ±9 remain sharp. At all extents of digestion, the smaller intracore bands 6 and 7 show enhanced intensity while the larger intracore bands (±9) are diminished (Fig. 3, scans 2 versus 3, 4 versus 5). Digests in 3 mM Mg²⁺ produce similar results (Fig. 3). Again, these specific changes are highly repeatable in different gels and nuclear preparations. Thus, polyamines (and Mg²⁺ at high concentrations) produce an alteration, mainly involving the large intracore bands, which affects both the amount and type of bands produced. The effects are quite different from the effects produced by increasing [NaCl] (Fig. 3, scans 4 versus 5).

I also examined low temperature digests (Fig. 3, lane 3). The only effect of lower temperatures is to slightly enhance the smaller intercore bands (Fig. 3, scan 3, ●). This effect is not as pronounced as that produced by very low salt.

**Staphylococcal Nuclease**

Since DNase I cleaves both spacer and core particle DNA simultaneously, the patterns produced contain information about both types of chromatin regions. On the other hand, staphylococcal nuclease cleavage is confined mainly to spacer, at least until quite late in digestion. Therefore, we can use staphylococcal nuclease digests to check conclusions about spacer DNA accessibility from the DNase I analysis.

Digestion under the standard conditions for staphylococcal nuclease, 0.05 mM Ca²⁺, produces a nucleosome repeat pattern (Fig. 4, lane 1). Digestion in 150 mM NaCl produces an even clearer nucleosome ladder, as judged by a lower interpeak background and the consequent ability to resolve more nucleosome repeat peaks (Fig. 4, lanes 1 versus 2). The background differences are quantified in densitometer scans of the higher resolution gel shown in lanes 3–6 (Fig. 4, scans 3 versus 4). The salt digests have generally higher peaks and lower interpeak valleys.

Interestingly, the internucleosome peak DNA produced in the very low salt digests occurs in a pattern of discrete bands rather than a smear. These bands run across the entire interpeak region and through the nucleosome peaks as well (cf. the I–II or H–III regions, Fig. 4, scan 3). The 150 mM NaCl digests show little evidence of such discrete banding, either in the nucleosome peak regions (Fig. 4, scan 4) or in extensive digests (not shown).

The increased interpeak DNA in low salt digests could result from an increase in the relative frequency of intracore cleavage or an increase in the amount of non-nucleosomal DNA. Very low salt conditions seem unlikely to produce more non-nucleosomal DNA than is present in 150 mM NaCl. For example, a DNase I ladder pattern, which is diagnostic of a DNA-histone interaction is most clearly present in very low salt. Occurrence in discrete bands rather than a smear also

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**Fig. 3.** Effects of multivalent cations and low temperatures on DNase I digestion patterns from yeast nuclear chromatin. Nuclear digests and electrophoresis were done as described under "Materials and Methods." Bands 8 and 11 in the intracore pattern are identified; bands in the intercore pattern are identified by ●. The numbers on the scans correspond to the numbers on the gel tracks. The unnumbered dashed scan is a digest in 3 mM Mg²⁺. Electrophoresis is from top to bottom in the photographs and from left to right in the scans. Digestion conditions: 0.5 mM Mg²⁺ plus 0.16 mM spermine/0.5 mM spermidine (tracks 1 and 4) or 150 mM NaCl (track 5); 30 °C (tracks 1, 2, 4, and 5) or 12 °C (track 3). The altered bands in the spermine/spermidine profiles are shown by × and their peak positions noted by dotted lines.
FIG. 4. Staphylococcal nuclease digestions of yeast nuclear chromatin under various conditions of digestion. Nuclear digestions were done as described under "Materials and Methods." The positions of di- (II) and tetranucleosomal length DNA (IV) in the gels or scans are shown. Electrophoresis is from top to bottom (photographs) or from left to right (densitometer scans). The numbers on the scans correspond to the same numbered gel tracks. The upper dotted line shows the pattern seen below the dinucleosomal peak in an extensive nuclear chromatin digest. Digestion conditions: tracks 1–6, 0.05 mM Ca\(^{2+}\), plus 150 mM NaCl (tracks 2 and 4) or 0.15 mM spermine/0.5 mM spermidine (track 6); track 5, 12 °C; tracks 1–4 and 6, 30 °C.

suggests a nucleoprotein origin for this DNA. Thus, the higher levels of interpeak DNA in low salt digests must reflect increased intracore cleavage. The lower interpeak DNA levels and clearer nucleosome repeat pattern in 150 mM NaCl must reflect a relative enhancement of spacer cleavage frequency versus intracore cleavage under those conditions. Thus, both staphylococcal nuclease and DNase I show enhanced spacer cleavage frequency in 150 mM NaCl.

These interpeak bands probably arise from endonucleolytic digestion rather than from exonucleolytic nibbling of already cleaved DNA for they remain clearly present in low temperature digests (Fig. 4, lane 5). Lower temperatures diminish the exonuclease activity associated with staphylococcal nuclease (Noll and Kornberg, 1977). The interpeak DNA also does not appear to result from premature "limit" cleavage, for the bands do not show the characteristic limit digest pattern of strong intensity maxima every 20 bp (Camerini-Otero et al., 1976). Instead, intensity falls off quite uniformly from the nucleosome peaks (Fig. 4, scan 3). Thus, the interpeak DNA must reflect a unique structure in very low salt chromatin. Limit type patterns can be seen below the dinucleosomal peak in very extensive nuclear digestes (Fig. 4, dotted line).

From digests in the presence of spermine/spermidine (or 3 mM Ca\(^{2+}\)), there is also considerable interpeak DNA but it is present as a smear instead of discrete bands, on the same gels as very low salt-digested samples showing discrete banding (Fig. 4, lanes 3 and 5 versus 6). The DNA in the nucleosome peak regions does not show discrete banding either (Fig. 4, lane 6).

**Gene-specific Yeast Chromatin**

Many of the same features noted in bulk chromatin are also present in the chromatin of individual genes. For example, the coding sequences of the ribosomal and galactokinase genes both show a typical DNase I ladder pattern (Lohr 1983a, 1983b), with relative intensities and sizes of intra- and intercore bands similar to those in hybridization profiles from bulk chromatin. However, chromatin from the region immediately upstream of the transcription initiation site of the 35 S ribosomal gene yields predominantly bands of the sizes of small intercore bands (cf. bands 6.5–10.5), with only very weak intensity at the positions of the intracore bands (cf. band 8, Fig. 5, lanes 1 versus 2). When the gene is less active, this region shows a more typical DNase I pattern (Lohr, 1984). Thus, understanding the features responsible for enhancing the smaller intercore bands in bulk chromatin digestes may help in understanding the chromatin structure in this functionally important region of the ribosomal gene.
The bulk chromatin staphylococcal nuclease profile differences between 150 mM NaCl and very low salt digests resemble the differences described previously (Lohr, 1983a) between inactive and active galactokinase single gene chromatin. On the inactive galactokinase gene, there is a clear nucleosome repeat pattern (Fig. 5, lane 3). When the gene is expressed, there is much more interpeak DNA present and it occurs in a pattern of discrete bands (Fig. 5, scan +), as in the low salt bulk chromatin digests. In both profiles, 15–17 bands can be resolved in the region from the mononucleosome to the dinucleosome peak (Fig. 5, scan + versus scan 0). Thus, active chromatin and very low salt bulk chromatin have structures which result in increased cleavage within the core particle, while inactive chromatin and bulk chromatin in 150 mM NaCl show an increased frequency of spacer cleavage and less intracore cleavage. These similarities suggest that the salt-dependent transition in bulk chromatin may provide a model for the active/inactive single gene chromatin structural changes noted in galactokinase and other genes (Prior et al., 1983; Levy and Noll, 1981; Lohr, 1984). It has not yet been possible to determine whether the salt-dependent DNase I digestion profile changes noted in bulk also occur in active/inactive galactokinase chromatin.

**Bulk Chicken Erythrocyte Chromatin**

DNase I profiles from yeast and chicken chromatins are similar up to band 11 of the intracore pattern but diverge above that point (Lohr et al., 1977a; Fig. 6). By analysis at higher resolution, we have determined that the two patterns come back into coincidence at band 16.5 (or sometimes at 17.5) and remain so until the gel loses resolution, in this case at least 10 bands =105 bp (Fig. 6). There is a slight misalignment around band 23.5 in these two particular traces, but this is not a reproducible observation. This extensive coincidence of patterns is a strong suggestion that the chromatin feature producing these bands from yeast must also be present in at

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**Fig. 5.** DNase I and staphylococcal nuclease digestion profiles from the yeast ribosomal and galactokinase genes. Tracks 1 and 2 show autoradiographic profiles from DNase I digests transferred to DBM paper (Lohr, 1983a) and probed with a small piece of DNA from the coding sequence (track 1, c) or from the upstream region (track 2, u) of the 35 S ribosomal gene. The intracore bands are located by ---; the intercore bands are located by ●. Tracks 3 and 4 show autoradiograms of the staphylococcal nuclease profiles from galactokinase chromatin when the gene is repressed (track 3, −) or expressed (track 4, +). The positions of mono- (I) and dinucleosomal (II) DNA peaks are shown. The positions of mono- (I) and dinucleosomal (II) DNA peaks are shown. Electrophoresis is from top to bottom (autoradiograms) or from left to right (densitometer traces).

**Fig. 6.** Comparison of the intercore band patterns in DNase I digestions of yeast and chicken erythrocyte nuclear chromatin. Nuclei were isolated, nuclease digestions were performed, and DNA was isolated and electrophoresed as described under "Materials and Methods." These patterns show yeast (a 0.5 mM MgCl$_2$ digest, Y) and chicken (a 150 mM NaCl digest, C) DNase I patterns analyzed on a high resolution gel. Bands 12 and 16.5 are located in the photograph and in the scan of the chicken track. Electrophoresis is from left to right. The topmost photograph shows a similar chicken digest on a lower resolution gel. Bands 12 and 25.5 are located by dashed lines (---) in both the low and high resolution chicken photographs.
least a fraction of chicken erythrocyte chromatin. In both chicken (topmost gel track in Fig. 6) and yeast (Fig. 1B), the regular band pattern continues beyond band 25.5, but I have not been able to compare these higher regions with enough accuracy to determine whether bands in the two patterns are coincident.

In yeast, the regularity of these intercore bands is a result of discrete length spacers, present in multiples of 10 nucleotides \( n = 0, 1, 2, \ldots \) (Lohr et al., 1977a). The precise sizes are thought to reflect the presence of a 5-bp increment in the spacer size, i.e. \( 10 \times n + 5 \) bp (Lohr and Van Holde, 1979). Thus, at least a portion of chicken chromatin also contains discrete length spacers with a 5-bp increment.

This data also show that a DNase I profile can be subdivided. In the intracore region and in the intercore above band 16.5, yeast and chicken are similar. In the transition region between bands 11 and 16.5, the yeast and chicken patterns differ. To learn more about yeast and chicken chromatin structure, I have compared the responses of these various parts of the DNase I profile to ionic strength changes.

**Intracore and Intercore Patterns**

Various conditions of digestion (very low ionic strength, moderate salt, presence of spermine/spermidine) produce some changes in the intracore region. The changes parallel those observed in yeast chromatin digests under the same condition, although they are usually less striking in chicken. For example, bands 10–12 are more prominent in salt-containing digests than in low salt digests (Fig. 7A, lanes 1 and 2 and scans 1 and 2). However, band 12 is always present, even in extensive low salt digests (Fig. 7A, lane 1). No small intercore bands (6.5–9.5) can be detected in the chicken chromatin digests, in either very low salt or low temperature profiles. These bands could occur at larger sizes, for example 13.5–16.5, because the chicken spacer is ~40 bp larger than the yeast spacer. However, there is no evidence of intercore bands in those regions either.

In the region above band 16.5, different conditions of digestion produce differences in the level of background DNA. As in yeast, the lowest backgrounds are in the very low salt digests. For example, the band clarity of the intercore pattern in lane 3 (Fig. 7A) approaches that in a yeast digest run on the same gel (not shown). The presence of salt produces higher backgrounds (Fig. 7A, lane 4) and thus decreased resolution of the intercore bands. It was initially suggested (Lohr et al., 1977a) that the chromatin feature producing the intercore pattern was not widespread in chicken chromatin, based on the low prominence of the intercore pattern in chicken digests. However, these digests were done in 150 mM NaCl. The ability to enhance the prominence of the chicken intercore pattern by digestion in low salt suggests that the chromatin feature producing this pattern must be present in a larger portion of chicken chromatin than originally suggested.

**Intracore/Intercore Transition Region**

In the region between bands 12 and 16.5, the presence or

![Fig. 7. Effects of ionic strength changes on DNase I digestion patterns of chicken erythrocyte nuclear chromatin.](image URL)
absence of salt affects the chicken profile strongly. For example, the next few bands above band 12 do not coincide in mobility in the 150 mM NaCl and the very low salt digests (Fig. 7A, scans 1 versus 2). These changes are shown in greater detail in the densitometer traces from a higher resolution gel (Fig. 7B). In 150 mM NaCl digests, the next bands above 12 have the approximate mobilities expected for intracore bands 13 and 14 (Fig. 7B, 150). In 350 mM NaCl digests, there is also a well resolved band 15 (Fig. 7B, 350) and one can reproducibly detect shoulders whose mobilities are approximately that of intracore bands 16 and 17 (Fig. 7B, V). Thus, in chicken, the presence of salt results in the appearance of new intracore bands, as well as enhancing the intracore bands normally present (bands 10–12). The presence of these new intracore bands causes the inter- to intercore transition to be more abrupt and the transition region smaller than in low salt (see below).

These new intracore bands often have slightly lower mobilities (larger sizes) than would be expected for bands in the intracore series; for example, band 15 can peak from 13.0–13.1 band numbers and band 14 can peak from 14.0–14.2 band numbers. The larger sizes occur more often in the 350 mM NaCl digests. It is not obvious why these bands are slightly larger than expected. Studies with isolated core particles show shorter separations between DNase I cleavage sites in the DNA near core ends (Lutter, 1979, 1981). These studies were not performed in 150 or 350 mM NaCl however. The presence of spacer DNA and other higher order structural features could also have an effect on the location of DNase I cutting sites near the ends of the core. There is increased separation between exonuclease III pausing sites in the end regions of the core particle (Prunell, 1983).

In low salt chicken digestions, there is generally a pattern of resolved bands above band 12, but mobilities of these bands differ from the mobilities of large intracore bands. Also, they are not all uniformly spaced; there is always one place in the pattern where two adjacent bands occur closer together than the normal spacing for DNase I bands. In the very low salt digestions (Fig. 7B, L0) and in 0.5 mM Mg\(^{2+}\) at low temperature (not shown), this usually occurs at the next band above band 12. In spermine/spermidine (Fig. 7B, S) and in 0.5 mM Mg\(^{2+}\) at 30 °C (not shown), the close spacing usually occurs after band 13. After this more closely spaced band, bands resume a larger and more regular spacing. In the very low salt digestions, which show the best resolution of this region, these bands are present at increments of ~0.9 band numbers, i.e. 13.8, 14.7, 15.6, and 16.5, after the first band at 12.7–12.8. The transition from intra- to intercore patterns is thus gradual and involves most of the region between bands 12 and 16.5. These features are quite reproducible in various digestions and gel systems. In 0.5 mM Mg\(^{2+}\) and spermine/spermidine digestions, bands are generally less well resolved and it is difficult to detect consistent behavior within this region. Despite these transition region differences, bands above band 16.5 are coincident in the yeast, the salt-containing, and the low salt chicken patterns.

The more closely spaced pair of bands in the low salt chicken digest is reminiscent of the close spacing of intercore band 11.5 and intracore band 11 in the yeast pattern, although the magnitude of the close spacing differs—0.7–0.8 in chicken versus 0.5 band number in yeast. The bands in this region of the chicken digest are quite broad. Thus, it is possible that the observed peak reflects a mixture of intercore (e.g. 12.5) and intracore (e.g. 13.0) bands and the sizes of the other transition region bands (13.8, 14.7, 15.6, 16.5) reflect an increasing intercore component in the peak at larger sizes.

The effects of ionic strength on staphylococcal nuclease digestions of chicken chromatin were also checked (not shown). The results are similar to yeast results. 150 mM NaCl favors spacer cleavage over intracore nucleosome patterns, while in low salt there is more intracore cleavage (more intranucleosome peak DNA). However, it is not possible to resolve a band pattern in the chicken low salt interpeak DNA. Also, the chicken nucleosome pattern remains clear and chicken nuclei do not show any signs of instability (lysis) in 350 mM NaCl. Digests were performed under other conditions, low temperature, and various divalent cations concentrations, but no noteworthy results were obtained.

**DISCUSSION**

**Bulk Yeast Chromatin**—This work confirms that basic features of yeast chromatin DNase I digestion profiles, such as the regular intercore pattern and the 5-bp offset between the inter- and intracore patterns, are present under a variety of ionic strength conditions. Thus, the structure producing these features must be the dominant and persistent structure in yeast chromatin at least at 150–350 mM NaCl.

Various ionic strength conditions do cause changes in the DNase I patterns. I suggest that these changes reflect alterations in chromatin structure, to which the nucleases are sensitive, rather than effects on the nucleases themselves, e.g. in their mode of attack, for several reasons. Firstly, the pattern changes are quite limited; under all conditions tested, most of the bands remain unchanged. Thus, in the production of most DNA fragments, DNase I must be operating in the usual way. The changes are confined to parts of the pattern which reflect digestion in regions of the nucleosome showing structural lability by other analyses (see below). Secondly, two different nucleases produce results which suggest the same types of changes (see below). Thirdly, many of the changes can be interpreted using features from nucleosomal structural transitions observed in the same ionic strength ranges in vitro (see below).

Most of the specific band changes in the yeast DNase I patterns involve the large intracore bands (10–12) and the small intercore bands (5.5–7.5). The former must arise mainly from sites near opposite ends of the core particle because their size approaches that of core particle DNA. Bands 5.5–7.5 reflect digestion at pairs of sites which between them total 30–50 bp into the core particle, since the average spacer length in yeast is 25 bp (e.g. 5.5 × 10.5 bp = 58 bp – 25 bp = 33 bp). Thus, both changes involve DNA near the ends of the core particle. However, the two types of changes occur under mutually exclusive conditions: one in the presence of salt (increase in bands 10–12); the other only in very low salt (increase in bands 5.5–7.5). Thus, a simple increase in accessibility of core end DNA is not a sufficient explanation.

There are also differences in spacer cleavage frequency; spacer cleavage is enhanced in 150 mM NaCl compared to very low salt digestes. These differences can account for the specific band pattern differences. Band patterns from DNase I digests reflect a competition of intracore cleavage, intercore cleavage, and spacer cleavage. A change in the relative frequency of any one of these affects the prominence of the others. For example, an increase in the frequency of spacer cleavage decreases the prominence of the intercore pattern, including small intercore bands, since a spacer cleavage destroys the possibility of generating any intercore bands from the two surrounding core particles. However, intracore bands can still be produced after an adjacent spacer cleavage and thus would not be diminished under these circumstances. Some could even be enhanced. For example, since large intracore bands are produced from cleavages in the same region of
the nucleosome as small intercore bands, enhanced spacer cleavage leading to a decrease in the latter (150 mM NaCl) could enhance the prominence of the former and vice versa (very low salt).

There are certainly other possible explanations for the band pattern changes. However, this one is favored because it is the simplest explanation for the data (all conditions, both nucleases) and it can even explain apparently contradictory observations such as the increased DNase I intercore cleavage but decreased staphylococcal nuclease intercore cleavage in 150 mM NaCl. Note that spacer cleavage frequency could change because spacer DNA becomes absolutely more (or less) accessible or because the frequency of competing cleavage processes changes or from a combination of the two. For example, in 150 mM NaCl, a decreased accessibility of core end DNA could contribute to the enhanced spacer cleavage noted.

The data and this interpretation suggest that end DNA is more sensitive to ionic strength than the more internal core DNA. This is consistent with a number of in vitro analyses of core particles (Weischem et al., 1978; McGhee and Pelsenfeld, 1980b; Uberbacher et al., 1983). End DNA has fewer higher-order DNA contacts (Shick et al., 1980) but as high a concentration of DNA phosphates as other parts of the core and thus should be most sensitive to ionic strength changes.

Spermine/spermidine effects also involve end DNA. The types of changes, blurring and peak size changes of the large intercore bands, suggest a possible loss of specific end DNA/histone contacts under these conditions. Spermine/spermidine may compete with histones for the weakly held end DNA. Preferential interaction of spermine/spermidine with spacer, another likely binding site for these cations, and core particle end DNA might be related to their ability to inhibit endogenous nuclease (Vanderbilt et al., 1982).

In vitro in low ionic strength, the core particle unfolds into a more extended structure (Wu et al., 1979; Liberti and Small, 1982). The transition involves relative histone movement (Burch and Martinson, 1980; Eshaghpour et al., 1980) or rearrangement (Uberbacher et al., 1985), but the same histone/core DNA contacts remain (Zayetz et al., 1982). Presumably, such a transition provides a way to relieve the increased repulsion between DNA phosphates in low salt, while still maintaining the DNA-histone opposite charge interactions, which are also stronger in low salt. Since 175-bp mononucleosomes (i.e., core particles plus extra DNA) unfold in the same way in the same ionic strength range (Uberbacher et al., 1983), this transition should also be able to take place in yeast nucleosomes (∼170 bp nucleosome repeat) in the nucleus.

The rearrangement of histones during such a transition could produce an increased core histone/spacer DNA interaction, either by exposing new regions of the histones to bind to spacer or increasing the extent of interaction of those histones which already interact with spacer in the folded core particle (Karpov et al., 1982; Richmond et al., 1984). Very low salt conditions would favor such interactions. The resulting increased nucleohistone character of spacer DNA should restrict its nucleosome accessibility and thus could produce the decreased spacer cleavage frequency noted in low salt. Histone/spacer DNA interaction can also explain the discrete bands in the regions which correspond to digestion in spacer DNA, the nucleosomal peaks in staphylococcal nuclease digests, assuming that discrete bands in a bulk chromatin staphylococcal nuclease digest reflect DNA/protein interactions, as is the case for DNase I (Kirkgaard and Wang, 1981; Rhodes and Klug, 1981). This seems likely.

The 150 mM NaCl and the very low salt chromatin should have distinctive higher order structures. In 150 mM NaCl, increased spacer cleavage frequencies suggest a chromatin fiber with an enhanced distinction between the spacer and the protected core particle domains. In very low salt, both the cleavage frequency and the cleavage products suggest a relatively more protected spacer and/or a relatively more exposed core, i.e., a fiber with a decreased distinction between spacer and core. The formation of this more uniform fiber in a stretch of nuclear chromatin should produce a higher order structure which is quite different from the well defined spacer/core alternation of the 150 mM NaCl fiber.

This more uniform low salt fiber could be related to the smooth fiber often detected in electron microscopy of active chromatin. If active chromatin unfolding resembles the low salt transition, modifications which increase the DNA repulsion terms, such as acetylation of lysines, should promote the unfolding and indeed, this correlation has been made (see review by Matthis et al., 1980). On the other hand, modifications which promote a more compact core (cf. histone deacetylation, DNA acylation, or even spermine/spermidine binding, etc.) could be associated with inactive chromatin.

Chicken Erythrocyte Chromatin—At least a fraction of chicken erythrocyte chromatin has spacers like yeast, discrete length with a 5-bp increment. Evidence for discrete length spacers has also been obtained in Drosophila (Karpov et al., 1982) and in rat liver (Strauss and Prunell, 1983). However, there is disagreement regarding the 5-bp increment in the spacer. Karpov et al. (1982) conclude that spacers are 10 × n bp in length. Their conclusion is based on the assumption of 150 bp as the appropriate size to use for the core particle in an exonuclease III digestion study. However, if one uses 147 bp as the appropriate size, then there is an increment of 6 bp, i.e., close to 5. Unfortunately, it is not clear what size to use to interpret these experiments. Thus, these observations are not compelling evidence against a 5-bp increment. Strauss and Prunell (1983) obtained digestion data consistent with a 5-bp increment but suggest that this increment arises from the variation in the angle of DNase I attack at the different DNase I cutting sites in the core particle, an effect postulated by Klug and Lutter (1981), rather than from an actual 5-bp increment in the spacer length. The available data are consistent with both interpretations.

The fraction of the chicken genome with discrete spacers may be substantial. The amount of DNA present in discrete bands in a DNase I intercore pattern gives only a minimum estimate because every spacer cleavage during digestion destroys all possibility for yielding intercore bands from the two adjacent core particles. The large spacer in chicken chromatin should present a large target for DNase I, resulting in significantly more protected spacer and/or a relatively more exposed core. The ability to enhance the prominence of the chicken intercore pattern suggests that discrete spacers may be a significant feature in chicken chromatin. In yeast, the prominent intercore pattern suggested initially that most or all of the chromatin had discrete spacers (Lohr and Van Holde, 1979). This was supported by the observation of intercore patterns in specific yeast genes, in both the inactive and actively expressed states (Lohr, 1983a, 1983b).

The forces responsible for stabilizing discrete spacer lengths may differ in yeast and chicken, even though the results produced are the same. For example, because of the small spacer and apparent rigidity of yeast chromatin (see below), packing constraints might be sufficient to ensure a discrete spacer. Because of the flexibility (see below) and the long length of spacer in chicken chromatin, additional features, perhaps involving H1 and H5, might be required to produce a discrete spacer. Their general occurrence suggests that the regularity in core particle arrangement reflected by discrete
length spacers may be important in cellular function.

The intracore/intercore transition regions and their responses to ionic strength changes differ markedly in chicken and yeast. It is not possible to interpret all the pattern features. However, one distinct part of the transition region in both organisms, large intracore bands, can be related to the DNA/core histone interaction. Intracore bands 11–14 reflect digestion near core particle ends; bands 15 and 16 reflect digestion in the chromatosome ends. The organism-dependent pattern differences involving these bands must reflect structural differences between the two chromatosins in these regions. For example, in 150 and 350 mM NaCl the chicken pattern is consistent (bands 15 and 16) with chromatosome structure (Simpson, 1978). However, the yeast pattern is not. Thus, yeast may not be able to form a chromatosome.

The more substantial changes seen in the chicken transition region patterns suggest that there is much more flexibility associated with the core end DNA region in chicken than in yeast chromatin. Both chromatins were analyzed under the same conditions and these various conditions do produce the same tendencies for change in both organisms. Yeast just does not respond as extensively as chicken.

Metazoan chromatin forms an extended 10-nm diameter fiber in low salt and a more compact 30-nm fiber in 150 mM salt (Thoma et al., 1979). Assuming that the same structures occur in the nucleus, the chicken DNaSe I results suggest that the organization of DNA on the ends of the core differs in these two fibers. In the 30-nm fiber there is a more extensive DNA/core histone interaction, reflected by the extension of the intracore pattern to higher bands, than in the low salt fiber. Termination of the intracore pattern at band 12 (=125 nucleotides) suggests that DNA/core histone interactions do not involve the entire length of core DNA in low salt. This may reflect the involvement of end DNA in the transition to the next particle in this more extended fiber. These conclusions are consistent with models for the structures of these fibers based on electron microscopy results (Butler, 1983).

Work on isolated core particles implicates the middle 100 bp, i.e. ~10 bands worth of DNA, as being the most tightly bound to the core (McGhee and Felsenfeld, 1980a).

Yeast forms higher order structures (Rattner et al., 1982) that resemble 30-nm fibers observed in other eukaryotes. The differences in yeast and chicken DNaSe I patterns suggest that either a 30-nm fiber can form in various ways (i.e. without folding more DNA on the core) or yeast forms a higher order structure which is not quite the same in detailed structure as the 30-nm fiber in metazoan chromatin. The (presumed) absence of H1 in yeast and the crucial role of H1 in forming the 30-nm fiber (Butler, 1983; Reeves, 1984) would seem to favor the latter alternative. Evidence of typical 30-nm fibers from other small repeat length chromatins (McGhee et al., 1983; Pearson et al., 1983) may not relate to yeast since there is H1 in those chromatins.

Thus, although the core particle is ubiquitous, it is probably the internal 100–120 bp which are truly constant. There are substantial organism-dependent differences in structural organization and conformational flexibility of the DNA on the ends of the core. These differences can produce different higher order structures.

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