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Organization of spacer DNA in chromatin

(nuclease/yeast/chicken erythrocytes/HeLa cells)

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ABSTRACT **Detailed analysis of the DNA fragment patterns produced by DNase I digestion of yeast, HeLa, and chicken erythrocyte nuclei reveals surprising features of nucleosome phasing. First, the spacer regions in phased yeast chromatin must be of lengths (10*m* + 5) base pairs, where *m* = 0, 1, 2, ... This feature is not seen in parallel studies of chicken erythrocyte chromatin. The 5-base pair increment in the yeast spacer imposes interesting restraints on the higher order structure of yeast chromatin. Second, we have been able to simulate the DNase I cutting patterns and get good agreement with the observed yeast patterns. Third, three different chromatins show a long range periodicity in the DNase I digest pattern, with a period half that of the staphylococcal nuclease repeat. These results suggest that the amount of chromatin observed in discrete extended-ladder bands is a *minimum* estimate of phasing and in fact phasing may be a more general feature.**

DNase I digestion of yeast, chicken erythrocyte, and HeLa nuclei produces "extended ladders" of bands of single-strand DNA fragments extending to well beyond the size of a nucleosomal repeat (1). This shows that the interparticle DNA, so-called linker or spacer DNA, occurs in discrete sizes in at least some fraction of the chromatin of these species. We have used the term "phasing" to denote this characteristic of spacer organization, because a discrete spacer requires that adjacent core particles be in a precise location relative to one another. Phasing, as used here, is not meant to suggest there is a precise location of a nucleosome with respect to a given DNA sequence. We present in this paper further observations on nucleosome phasing that provide at least partial explanation for many of the details of DNase I cleavage patterns in yeast and other eukaryotes.

EXPERIMENTAL PROCEDURES

HeLa, chicken erythrocyte, and yeast nuclei (the last from growing and stationary cells) were isolated as described (2, 3). DNase I digestion and DNA extraction were performed as in ref. 2. DNA was electrophoresed on either 5.5% or 8% polyacrylamide/98% formamide gels as described (4) or 8, 12, 13, or 15.5% polyacrylamide/urea gels prepared as described (5). Gels were analyzed as in ref. 1.

RESULTS

Quantitating Spacer in Phased Chromatin. In Fig. 1 we show gel photographs and scans of the "extended-ladder" pattern obtained by DNase I digestion of yeast and chicken erythrocyte nuclei. There is an anomalous region in gels of DNase I digests of yeast nuclei in which the regular pattern of bands observed above 140 bases (b) and below 110 b breaks down (1). The pattern appears as if there are really two series of bands with a regularly spaced series of bands above 140 b that is out of phase with the regularly spaced series of bands below 110 b to produce closer spacing of bands in the region where the two series overlap. In fact, a plot of band number vs. gel mobility shows precisely this behavior (Fig. 2). Both series are quite regular; however, in the overlap region there is a transition from the "low" (10–≈110 b) pattern to the "high" (>140 b) series.

We have accurately calibrated the band sizes of the yeast and chicken erythrocyte DNA and of our PM2 *Hae* III restriction fragments against ϕX174 *Hae* III, simian virus 40 *Hae* III, and pBR322 *Hae* III restriction fragments, whose sizes are exactly known from DNA sequence determinations (6–8). Results are shown in Table 1. Notice that (*i*) in the overlap region the two patterns differ by about 4–6 b, quantitatively showing the amount of DNA by which the high and low series of bands are out of phase and (*ii*) clearly the interval between bands in both the low and high series slightly *exceeds* 10 b.

A graphical representation of the size data (Fig. 3) shows a clear discontinuity in the sizes of the two series of bands obtained by DNase I digestion of yeast nuclei. The lower series can be fitted accurately by $N = 10.5n$, where n is the band number and N is the size in bases. The upper series is fitted by $N = 10.5n - 5.0$. The value of 5.0 has been obtained from Fig. 3 *inset*. This data shows quite clearly that the upper series of bands is displaced by 5 b from the lower set. Because the lower series of bands comes predominantly from intracore DNase I nicks whereas the higher series of bands must come from nicks in adjacent nucleosomes, but presumably at the usual intracore sites, the principal difference between these two series is the presence of spacer DNA in the high series. Thus, we conclude that spacer sizes must be (10*m* + 5) base pairs (bp) in size (*m* = 0, 1, 2, . . .). In other words, core particles in yeast chromatin must be spaced by approximately $m + 0.5$ turns of DNA.

The same discontinuity and DNA fragment sizes are observed for both growing and stationary-phase yeast. Interestingly, digestions of neither the chicken erythrocyte nuclei nor the compact dimer (10) show either the close spacing of bands in an overlap region (Fig. 1) or the discontinuity associated with the presence of two offset series of bands (Figs. 2 and 3). We cannot entirely exclude the possibility that a discontinuity occurs at larger DNA sizes in the chicken erythrocyte nuclear digests (see upper part of Fig. 3), because these larger sizes are hard to measure with sufficient accuracy due to the fact that DNase I digests of chicken erythrocyte nuclei exhibit a background that obscures the bands (see Fig. 1). The bands are also more closely spaced in this size range.

The size differences of the DNase I bands and the lack of a yeastlike "skip" in the chicken compact dimer DNA pattern make it very unlikely that a previous suggestion—phasing in yeast arises from a population of "compact" oligomers (10)—is correct. A more plausible explanation is given in *Discussion*.

Analysis of Yeast Digestion Pattern. The presence of a 5-bp increment in the spacer suggests that there should be bands between at least some of the <140-b bands, arising from di-

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Abbreviations: bp, base pair; b, bases of single-stranded DNA.

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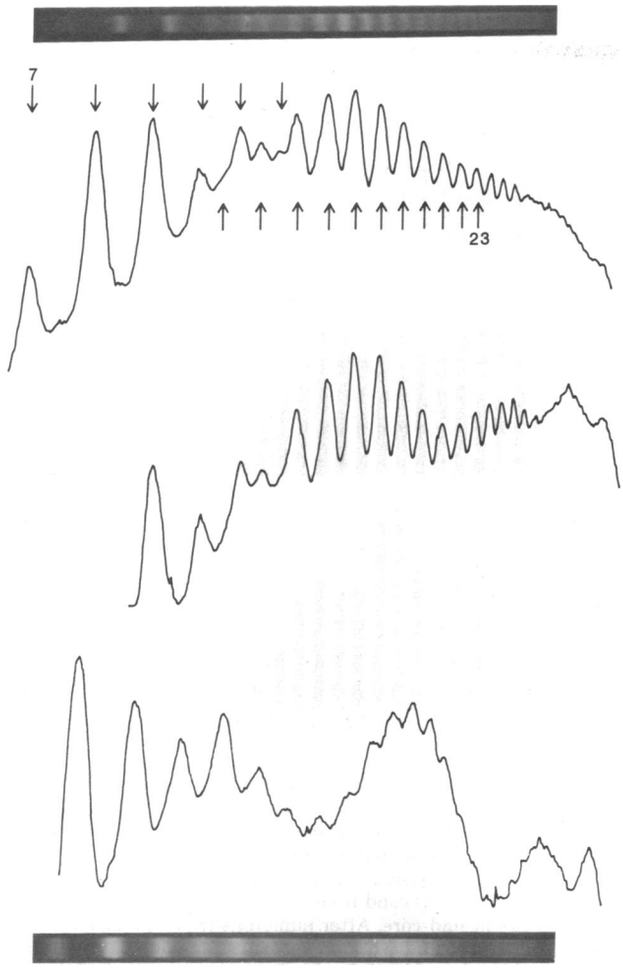

FIG. 1. Single-strand DNA fragments produced by DNase I digestion of nuclear chromatin from: (*Top*) Growing yeast (photograph and scan). (*Middle*) Stationary phase yeast (scan). (*Bottom*) Chicken erythrocytes (scan and photograph). Band positions are denoted by arrows and bands 7 and 23 are labeled. Because bands 1–5 have been run off of the gels, we have used the characteristically intense band at about 80 (band 8) as the reference point. Samples were electrophoresed on 8% polyacrylamide/7 M urea denaturing gels. Direction of electrophoresis is from right to left. The alignment of photographs and adjacent scans is intended to be only approximate.

gestions at the frequently cut sites across one spacer—for example, from site 13 in one core to site 5 in the next. Thus, there should be two series of bands at sizes <100 b, one from *intra*core nicks and the other from short *inter*core nicks. However, the latter series is not normally seen in DNase I digests, presumably because these bands are generally weak. This can be quantitatively shown by computer simulation of the digestion patterns (Fig. 4). In general, the predicted patterns are in excellent agreement with experimental patterns [compare Fig. 1 (growing and stationary yeast) to Fig. 4]. Up to $n = 10$ or 11, the "intracore" bands clearly dominate. Even though there are contributions in this region from the other band set, they will be difficult to resolve for the following reason. A weak band lying between two strong bands must have a certain minimal intensity before it will be discernible as a maximum. For band series of the kind shown in Fig. 1, we calculate that an interleaved weak band must be about half as intense as the flanking bands before it appears as a maximum. As Fig. 4 shows, most of the intercore bands below $j = 10$ will therefore be invisible under these conditions.

We have looked for evidence of the presence of small intercore bands on very-high-resolution gels (Fig. 5), in which the

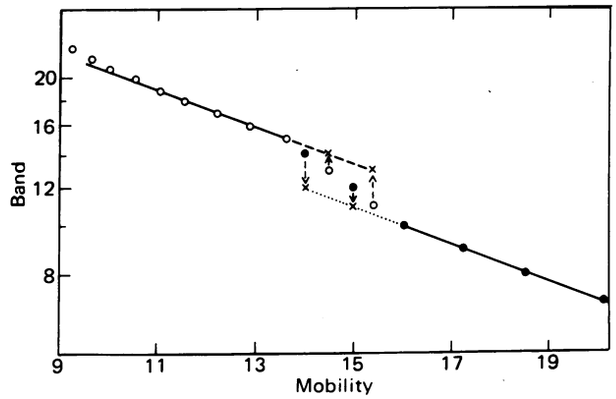

FIG. 2. A graph of band number vs. mobility for the yeast nuclear digest (Y_G) shown in Fig. 1. Each observed band up to band 23 has been counted. The arrows show that bands in the transition region, which do not fall on either line, can be assigned to the lower series (●) or the upper series (○) by changing the assigned band number by an increment equal to the number of intervening bands between band X and the last band, which does fall on a line. Thus, a change of one or two bands puts the transition points in line with either the upper or lower series.

increased resolution might allow us to see these weak bands. As expected, only yeast shows any evidence of these bands and, as predicted from Fig. 4, the bands are only visible between larger intracore bands—i.e., 5, 6, and 7.

Returning to the predictions of Fig. 4, we see that above band 13, the intercore series dominates the pattern. Another weak set of bands (from next-nearest-neighbor cuts) appears at the high end, but these will generally be negligible. In the middle region (between about $j = 10$ and $j = 13$), both intercore and intracore bands are present at about equal strength, as observed in Fig. 1. In short, the entire pattern can be accurately pre-

Table 1. Sizes of yeast and chicken erythrocyte DNA fragments produced by digestion of nuclear chromatin by DNase I

Yeast, b	Chicken erythrocyte, b	Rat liver, b
53.4 ± 0.8 (4)	52 (2)	51.9
63.2 ± 0.7 (5)	63 (2)	62.9
72.7 ± 0.4 (5)	72.6 ± 0.5 (3)	72.5
83.7 ± 0.4 (6)	82.6 ± 0.6 (4)	82.7
94.9 ± 0.8 (6)	93.6 ± 1.0 (4)	93.4
105.4 ± 1.1 (6)	103.4 ± 1.2 (5)	103.0
115.4 ± 1.0 (6)	113.8 ± 1.2 (5)	113.4
125 (1)	124.2 ± 1.8 (5)	123.9
110 (1)	133.3 ± 0.9 (4)	
121.2 ± 0.7 (6)	142.0 ± 1.3 (4)	
132.1 ± 0.4 (6)	154.4 ± 1.5 (5)	
142.7 ± 0.8 (6)	165.9 ± 1.6 (5)	
152.7 ± 0.8 (6)	176.1 ± 2.1 (5)	
163.4 ± 0.5 (6)	183.8 ± 1.9 (5)	
173.5 ± 0.6 (6)	194.2 ± 2.2 (5)	
184 ± 0.7 (6)	202.2 ± 2.2 (5)	
194.8 ± 0.3 (6)	212 ± 2.5 (3)	
205.3 ± 1.3 (6)		
215.5 ± 0.7 (3)		

The number of independent determinations (different digestion times and different 8% polyacrylamide/formamide gels) for each size is in parentheses. An independent determination consisted of a calibration of the digestion fragments against one of the primary standards (ϕX174, simian virus 40, or pBR322) run in a directly adjacent well in a region of the gel where no curvature was present. The average of all these independent determinations yields the size ±SD shown. Rat liver sizes are from Prunell *et al.* (9). The values for yeast fragments are divided by a dotted line to reflect the two series of bands observed.

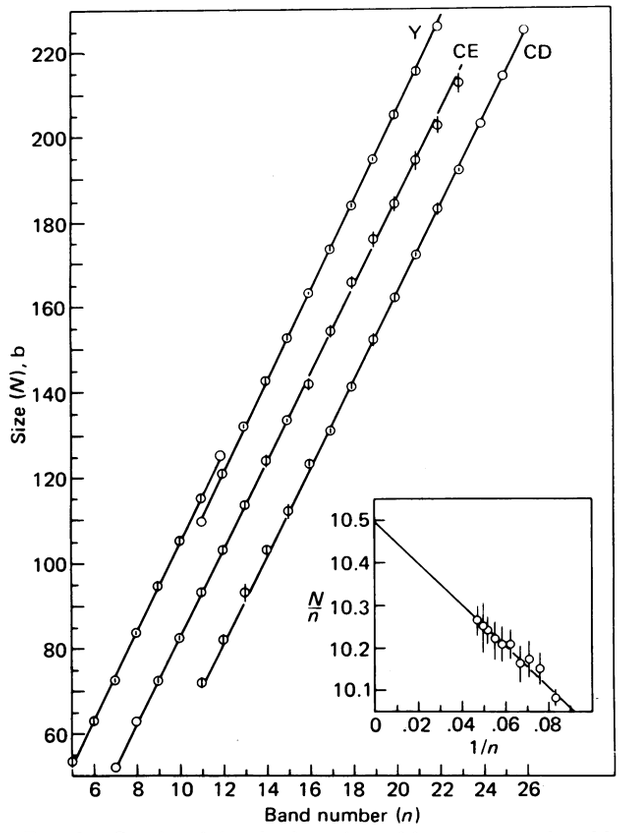

FIG. 3. Graphs of the absolute sizes of fragments produced by DNase I digestion vs. band number. Band numbers were counted from the prominent band 8 in each series and numbered consecutively. Lines Y, CE, and CD correspond to fragments from yeast nuclei, chicken erythrocyte nuclei, and chicken erythrocyte compact dimers (10), respectively. The band number (n) scale (abscissa) is for yeast; band numbers for CE and CD have been increased by 2 and 4, respectively, to displace the lines for clarity. Band numbers above 10 in this figure and Fig. 1 do not correspond because it is possible to resolve more bands in the overlap region on urea gels than on formamide gels. The average deviations for all points corresponding to multiple determinations are shown by the vertical lines; points without bars correspond to a single observation. (*Inset*) Quantitation of the "skip" in the yeast series, according to the equation $N/n = r - d/n$, where r is the number of bases between nicking sites and d is the displacement between the two series. The points shown are for the upper series of yeast fragments; bars indicate SD of fragment sizes. Slope = 5.

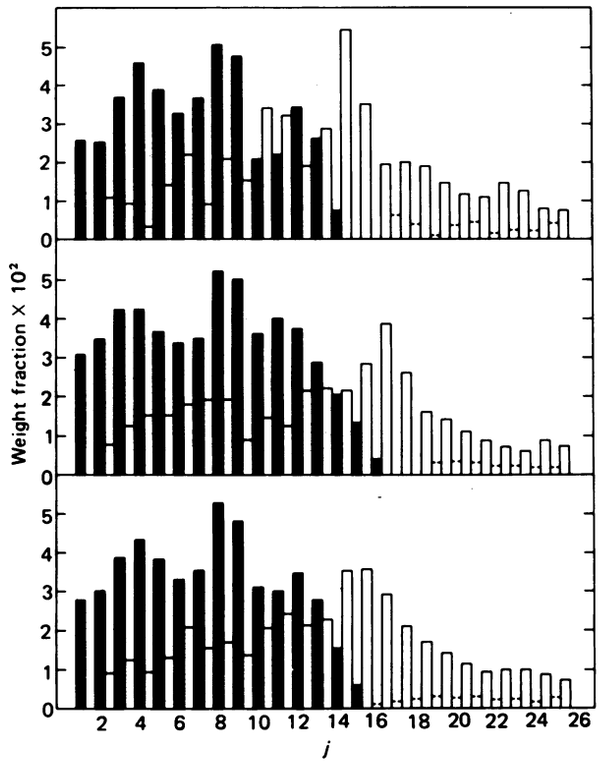

FIG. 4. Simulation of DNase I nicking patterns. The mathematical details and the computer program we used are available on request. To predict the nicking patterns for yeast chromatin, we assume, first, that nicking frequencies obtained by Lutter (11) from rat liver core particles apply to yeast chromatin. All data shown correspond to Lutter's (11) conditions at a 30-sec digestion except for the cleavage site at mid-core. After numerous trials we have taken the value for the probability of nicking at Lutter's point 70 to be 0.155 instead of his value of 0.067 for this digestion time. We find that with chromatin digestion, band 6 is exceptionally weak when Lutter's value is used. Second, because we do not know the cutting probabilities at spacer sites, we tried different values for the spacer nicking frequency. We have used the probability of a particular spacer link to be hit as 0.25; this is intermediate between the most and least frequently nicked internal sites. Using a larger value leads to very rapid depletion of the upper series of bands. For ease of calculation, our program assumes that DNase I cleaves at specific sites in the spacer. Data presented later in this paper suggests that this is probably not correct, but the main effect of nonspecific nicking should be to contribute to background in the pattern. Finally, we do not know the precise length of spacer DNA in that portion of the yeast chromatin that gives rise to the extended ladder. We have tried values of n = 14, 15, and 16 (corresponding to repeat lengths of about 152, 163.5, and 173 bp, respectively). (*Top*) Results for n = 14. (*Middle*) Results for n = 16. (*Bottom*) Average results for an equal-weight mixture of n = 14, 15, and 16. In each case the weight fraction (the same quantity that is measured by ethidium bromide staining of DNA in gels) is graphed vs. band number (j). Fragments from intracore nicks are shown by shaded bars and those that cross one linker are shown by unshaded bars. The latter have been displaced upward by 0.5 j unit, corresponding to a 5-b increment in the linker. The broken bars to the right correspond to fragments that cross two linkers; they have been shifted upward by one j unit.

dicted, including such details as the overlap region between the intercore and intracore series and the lack of significant intercore bands $j < 10$.

Long-Range Periodicity in DNase I Patterns. DNase I shows some tendency to produce nucleosome-length repeats of DNA (2). However, when visualized on the appropriate gel (Fig. 6), this pattern becomes quite striking. Whereas staphylococcus nuclease digestion of chromatin produces mainly nucleosome repeat-length DNA fragments, reflecting the tendency of this enzyme to preferentially digest between core particles along the chromatin strand, spleen DNase II can be induced to recognize both the spacer and a site approximately in the middle of the nucleosome to produce a set of DNA fragments that are multiples of a basic size, one half of the staphylococcal nuclease repeat size (12). The pancreatic DNase I patterns resemble the DNase II patterns (12).

Whereas the simulated digestion patterns (Fig. 4) can generate a weak half-repeat pattern, such as in yeast (Fig. 1 *top*), we have not been able to simulate the intense half-repeat patterns seen for chicken erythrocyte or HeLa digests. Furthermore, if the increased intensities in the repeat region were due merely to local increased DNA concentrations of the extended ladder bands in these regions, these bands should be more clearly resolved from the background and show a higher band height-to-valley ratio than bands from the interpeak regions. They do not. Thus, another explanation is needed. If DNase I nicks strongly at a midnucleosome site, as DNase II can, then a combination of random spacer and specific midnucleosome attack will yield a repeat pattern of fragments spaced at half multiples of the staphylococcal nuclease repeat size. The attack

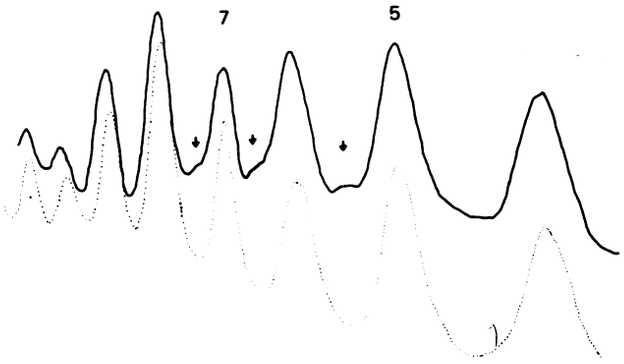

FIG. 5. High-resolution analysis of DNase I digestion products. DNA from DNase I digests of yeast (—) and chicken erythrocyte (·····) intranuclear chromatin and chicken core particles was end-labeled with ^{32}P, using [^{32}P]ATP and polynucleotide kinase, and electrophoresed on 12, 13, and 15% polyacrylamide/urea gels, which were then autoradiographed and scanned. On the higher-percentage gel, the lower intercore bands (between $j = 1$ and $j = 3$) could not be detected in yeast or in chicken digests (not shown). By running the smaller DNA (<30 b) off the gel, it is possible to detect intercore bands between intracore bands 5, 6, and 7 in yeast but not in chicken erythrocyte or in core particle digests (not shown).

seems to occur randomly throughout the spacer because the half-repeat peaks consist of continua of DNA sizes, even on high-resolution gels in which the extended-ladder bands are quite clearly resolved (see Fig. 1 *bottom*). Random cleavage within the spacer is certainly not incompatible with discrete spacer sizes and, in fact, is much easier to understand than specific spacer cleavage sites.

This suggestion immediately explains a number of puzzling observations about phasing. Random spacer cleavages combined with the many internal core particle cleavage sites can produce a great heterogeneity of DNA sizes, which is responsible for the extensive background in extended ladder patterns of most chromatin. The clarity of an extended ladder will thus depend on the relative rate of DNase I attack of spacer vs. core particle. DNA with larger spacers, such as in the chicken erythrocyte (≈60 bp), should show more spacer attack and hence would necessarily show a less clear extended ladder than

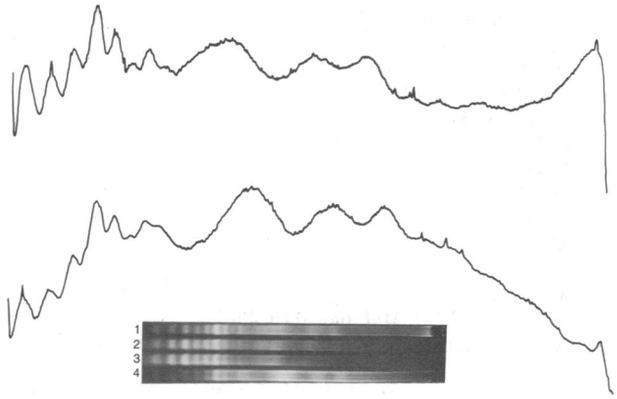

FIG. 6. Low-resolution gel electrophoresis of DNA fragments produced by DNase I digestion of HeLa (upper curve) and chicken erythrocyte (lower curve) nuclei. Below is shown the gel (5.5% polyacrylamide/formamide) from which the scans were made. Lanes: 1–3, HeLa nuclei digested to various extents; 4, chicken erythrocyte nuclei. Electrophoresis is from right to left. From a number of such gels, we find average peak sizes at 200 b, 304 b, 397 b, 511 b, and 619 b (about 101 b for the chicken erythrocyte DNase I repeat) and at 175 b, 269 b, 360 b, 454 b, and 547 b (about 90 b for the HeLa DNase repeat). In yeast, the DNase I repeat is only weakly detectable, mainly in digests of nuclei from stationary cells, but it stills shows values of one-half of the staphylococcal nuclease repeat.

yeast, which has a small spacer (≤25 bp). This is observed (1). However, *spacerless* chromatin must produce a background-free ladder with no evidence of the long-range DNase I repeat observed in chicken nuclei. This is the case (1). Thus, phasing may involve a much larger fraction of the chromatin than previously suggested (1), because much of the background arises not from a lack of phasing but from the possibilities of random DNase I nicks in the spacer.

This explanation needs one *ad hoc* assumption: a strong midnucleosome nicking site. The DNase I nicking site map determined from digestion of end-labeled core particles does not show a frequently nicked site in the middle of the core particle (11, 13, 14). However, to satisfactorily simulate different features of the extended ladder pattern of yeast nuclei, it was necessary to markedly increase the probability of cutting at the central site (see legend to Fig. 4). Analogously, the spleen DNase II map does not show a strong site in the middle of the core particle (15), but cleavage does occur readily in the middle of the nucleosome in intact chromatin (12). Even oligonucleosomes redigested with either DNase I (data not shown) or DNase II (12) do not show the clear half-repeat patterns observed with intact chromatin. These seeming contradictions could be resolved if intact chromatin is in a conformation that exposes an internal core particle site to nuclease attack. The availability of the central site would depend on and thus reflect internucleosomal interaction.

DISCUSSION

Probably the most surprising result of this work is the detection of a 5-bp increment in the yeast spacer. Whereas repeat length seems to be variable along the yeast chromatin strand (2, 4), the observation of bands larger than one repeat length in DNase I digests (1) proves that spacer DNA occurs in discrete lengths. Combining all these observations, we conclude that quantized spacers in yeast are $(10m + 5)$ bp in length, where $m = 0, 1, 2, \ldots$.—i.e., 5, 15, or 25 bp. We know of no accurate way to estimate the relative abundance of the various spacer lengths. However, from the absence of bands $10.5m$ in length above $n = 13$, we know that there are virtually no spacers $10m$ bp long.

The presence of a 5-bp increment in the spacer has some interesting structural implications. The core particle itself contains an integral number of DNA turns: 10.5 bp per turn × 14 turns = 147 bp (≈ 148 bp was measured for the yeast core particle by using the recalibrated PM2 *Hae* III fragments as standards). If DNA begins to wrap around each core particle with a particular orientation on the histone core, then the 5-bp increment will have the effect of reversing the strand orientation in adjacent nucleosomes. Thus, if one strand has a particular orientation upon entering one core particle, the other strand will be in the same orientation on the next core particle.

However, the most interesting consequence of the 5-bp increment arises in the geometry of core particle arrangement along the chromatin strand. DNA is thought to be wound in a left-handed coil around the core particle (16). Again, if there is a definite orientation of DNA with respect to the histone core [i.e., the major groove facing in or out at the entry point; see figure 1 of Carter (17)], then there arises a severe constraint on the way adjacent nucleosomes are arranged in space. Work with models has shown us that one cannot stack two neighboring nucleosomes separated by $10m + 5$ bp spacers on top of one another with histone faces (the ≈100-Å-diameter surfaces) parallel and have DNA in both be coiled left-handed. Rather, they must be arranged in a side-to-side manner, as shown in Fig. 7, and can neatly pack together. As far as we are aware, there is no evidence against such a side-to-side chromatin fiber.

In this model there can be interaction of DNA in one core

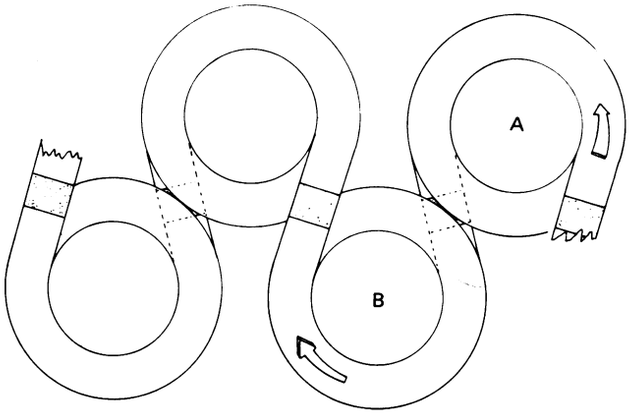

FIG. 7. A schematic drawing of the kind of chromatin structure imposed when spacer lengths are ($10m + 5$) bp. For simplicity we show a structure with a 5-bp spacer (dotted) and coplanar nucleosomes, although tipped orientations are possible, perhaps even more likely. The DNA enters core particle A at the top, descends for 1.75 turns, crosses to particle B via the spacer, and ascends for 1.75 turns. The sequence then repeats; thus, the two nucleosomes constitute a structural repeating unit. The core particles are drawn as 112-Å (outer diameter) cylinders with 22-Å-diameter DNA. For these to be in contact, about 7 bp of DNA must be loosened from the core at each end. Both compact and more open structures of the same type can be generated by using longer (15 bp, 25 bp) spacers or changes in the amount of DNA loosened from the core. We would expect, on the basis of thermal denaturation studies (18), that amounts up to 20 bp at each end could be so loosened.

particle with core particle histones in adjacent cores. Such elements of long-range interaction are probably necessary to generate stable higher-order structures. In addition, there are elements of symmetry inherent in the model; there is a pseudodyad axis passing through the spacer in a direction perpendicular to the core particle face (as drawn in Fig. 7). If the particles are tilted, the axis bisects the angle formed by the perpendicular to the faces. Thus, symmetry exists between nucleosomes (as well as within nucleosomes) to form a structure that repeats every second nucleosome.

We should point out that one can coin-stack core particles (one on top of another) separated by $10m + 5$ bp spacers if the twist of the DNA supercoil on the core particle changes from right-handed to left-handed every other nucleosome. However, because such a structure has zero net supercoiling, we rejected it.

The number of base pairs per turn is also of interest. Careful analysis of the slopes of the lines in Fig. 3 gives values of 10.5 for yeast but 10.3–10.4 for chicken, in agreement with the numbers determined by other workers using other multicellular eukaryotic systems (9, 19). Sizes of the chicken erythrocyte nuclear DNase I bands agree with the precisely determined sizes of the rat liver DNase I bands (9), but the yeast sizes do not (Table 1), corroborating the slight but measureable differences observed in yeast. The nonintegral number of base pairs per turn means that the average repeat must be a mixture of different values, for example 10 and 11 bp per nicking site. Thus, yeast chromatin is slightly underwound relative to chicken chromatin. This may be associated with the high transcriptional activity of yeast (cf. ref. 3).

The observation of the half-repeat pattern in the DNase I digests (and an accessible site at midnucleosome) generalizes the observation of a half-repeat with spleen DNase II. This pattern is not just a reflection of the peculiarities of a specific enzyme, but must reflect a modification in the structure of nucleosomes organized in the nuclear structure. This and the fact that the yeast DNase I fragments show a quite different pattern of digestion and slightly different pitch than is obtained from chicken erythrocyte or HeLa nuclei indicate that accessibility of nuclease cleavage sites is in some sense determined by nucleosome structure and is not just a reflection of some property of DNA itself.

The amount of chromatin involved in phasing has always been unknown. Based on background throughout the gels, we originally concluded that only a portion of yeast chromatin and even smaller portions of HeLa and chicken erythrocyte chromatin are phased. However, the evidence for random spacer digestion by DNase I suggests that the amount of DNA present in sharp, extended ladder bands is a *minimum* estimate of the amount of phased chromatin. In fact, we cannot exclude the possibility that all the chromatin in yeast and even in chicken erythrocytes is phased and that the background differences are due merely to differential but nonnegligible rates of spacer attack.

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