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Detailed Analysis of the Nucleosomal Organization of Transcribed DNA in Yeast Chromatin†

D. E. Lohr

ABSTRACT: The precise chromatin structure of actively transcribed DNA in yeast has been analyzed by electrophoretic transfer of high-resolution staphylococcal nuclease and DNase I chromatin digest DNA patterns to DBM paper and hybridization with active sequence probes. The DNA patterns of the transcribed DNA sequences resemble the DNA patterns produced by digestion of bulk yeast nucleosomes. Hence, these active sequences must be arranged in "typical" nucleosome structures. Furthermore, in details of the structure, the active sequence nucleosomes look almost exactly like the average yeast nucleosome in repeat length, in the length of DNA associated with the core particle, in the amount and type of heterogeneity found within and between the oligomeric and monomeric repeat lengths of DNA, in the occurrence of discrete spacer lengths including the characteristic five nucleotide increments (i.e., 5, 15, 25, ... base pairs), and in the length of DNA between yeast nucleosomes. Early in digestion, there are some differences: increases in peak breadths (i.e., in the distribution of spacer lengths) and some preferential release of monomer DNA. These results suggest that transcribed DNA can exist in the typical (yeast) type of nucleosome organization and thus that active chromatin regions do not necessarily require profound structural rearrangements. The slight differences noted are consistent with some slight, mainly spacer, modification in the vicinity of the transcription event itself.

The basis of the selectivity of eukaryotic transcription and its relationship to the presence and the properties of nucleosomes remains unknown despite intense study (Mathis et al., 1980). The current model, largely on the basis of work with chicken globin and ovalbumin (Mathis et al., 1980), envisions that active genes do in fact contain nucleosomes whose structure is subtly yet distinctly altered with respect to nonactive nucleosomes, an alteration which, for example, is reflected in an increased susceptibility to DNase I digestion (Weintraub & Groudine, 1976).

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An understanding of many aspects of bulk nucleosome structure originated from precise analysis of the details of the DNA patterns produced by staphylococcal nuclease and DNase I digestion of chromatin: repeat length, spacer length, amount of DNA in the core particle, heterogeneity of spacer lengths, details of the conformation of DNA within a core particle, heterogeneity in types of nucleosome structures, and "correlation" (phasing) in the spacing of neighboring nucleosomes. However, much of our knowledge of the chromatin structure of active nucleosomes is "kinetic" information, i.e., data on the rate at which a sequence (Weintraub & Groudine, 1976) or set of sequences (cf. Levy W. & Dixon, 1977) is selectively digested out of the genome, often to uncharacterized products. Unfortunately, data which yield "structural" information, such as DNA peak sizes, peak widths and shapes, interpeak profiles, and intensity levels relative to peak levels for staphylococcal nuclease and DNase I digestions of active genes, is not available at the same level of precision as for the bulk sequence nucleosomes.

Recent developments in gel techniques, electrophoretic transfer of DNA (Stellwag & Dahlberg, 1980) onto covalently binding DBM paper (Alwine et al., 1980), suggest a way to try to remedy this dearth of structural information on active nucleosomes. I modified these techniques to achieve efficient transfer of DNA to covalently binding DBM paper from the high-resolution gels needed to see the fine structure in nuclease digest patterns. This approach permits structural analysis of the nucleosomal arrangement of any given sequence or set of particular sequences at the same level of detail as is possible for bulk sequences.

As a first application of the method, I have chosen to look at two sets of "active" sequences: (1) complementary DNA synthesized from poly(A)-containing mRNA, and (2) in vitro synthesized RNA, elongated in isolated nuclei. The cDNA probe looks at the structure of *transcribed* chromatin, that is, sequences expressed at some time during the cell cycle and thus existing in the total poly(A+) mRNA population. One would also like to analyze *transcribing* chromatin, that is, sequences being expressed at any given moment in the life of the cell. Analysis of this state is at present unattainable. The latter probe, in vitro RNA, offers a beginning step toward analysis of this state. Use of these two heterogeneous active sequence probes is intended to ask whether there is any unique feature of nucleosome arrangement on active sequences which is *generally* true for all active sequences. Subsequent work will analyze individual active DNA sequences.

Experimental Procedures

Yeast cells were grown, nuclei were isolated, staphylococcal nuclease and DNase I digestions were performed, and DNA was extracted by previously described methods (Lohr et al., 1977a,b).

Staphylococcal nuclease digested DNA was electrophoresed on composite acrylamide (3.0–4.5%)/agarose (0.5–0.7%) slab gels at 6 V/cm. DNase I digested DNA was electrophoresed on composite acrylamide (5.5%)/agarose (0.4–0.6%) slab gels containing 7 M urea at 10 V/cm.

The DNA patterns in these gels were electrophoretically transferred for 6–12 h at 4 °C by using an E-C gel destainer as described by Stellwag & Dahlberg (1980), at 10 V/cm in 40–50 mM sodium phosphate, pH 5.5, to DBM paper prepared exactly as described by Alwine et al. (1980). DBM papers containing transferred DNA patterns were prehybridized and hybridized at 42 °C, using buffers described by Alwine et al. (1980). The stringent wash at 50 °C in ~0.1 × SSC often could be extended to 1–3 h to remove background. The use of these rather stringent wash conditions ensures that low homology hybridization of probe to bound DNA is minimized. Autoradiography was done at −80 °C by using Kodak XR5 film and Du Pont Lightning Plus intensifying screens. After autoradiography, the hybridized probe was removed from the paper by incubation in 0.4 N NaOH at 37–42 °C for 30 min to 2 h until the hand monitor showed only background levels of probe remaining on the paper. The paper was then washed extensively with H_2O. Occasionally pieces of paper had to be put aside for a few weeks because all probe could not be removed. Some pieces of paper have been put through six or seven cycles of wash and rehybridization with no apparent loss of hybridization efficiency.

Several types of ^{32}P probes were used:

(*a*) *Kinased Total.* Total yeast nuclear DNA was sonicated and treated with agarose-immobilized calf alkaline phosphatase for 1 h at 37 °C in 20 mM Tris (pH 7.9), 50 mM NaCl, and 10 mM Mg^{2+}. The phosphatase was centrifuged out, DNA was boiled, ≃0.1 volume of 10 × kinase buffer (500 mM Tris, pH 7.2, and 100 mM Mg^{2+}) was added, and the mixture was diluted by addition of an equal volume of 1 × kinase buffer. This mix was used to dissolve dried-down [^{32}P]ATP. β-Mercaptoethanol to 50 mM and 5–10 units of T_4 polynucleotide kinase were added, and the reaction was allowed to proceed for 1 h at 37 °C.

(*b*) *cDNA.* Total yeast RNA from logarithmically growing cells was isolated by standard phenol extraction techniques and then treated with DNase I which had been freed from residual RNase by affinity chromatography on 3′,5′-diphosphouridine (Maxwell et al., 1977). The RNA was passed over an oligo(dT)–cellulose column in 0.4 M NaCl, and bound RNA was eluted in low salt exactly as described by Hereford & Rosbash (1977). This bound RNA was heated to 60 °C for 5–10 min, cooled, and then rerun on oligo(dT)–cellulose as before. The second bound fraction was used as the template to make complementary DNA. The usual yield of poly(A+) mRNA from total was 1–2%. cDNA was synthesized exactly as described by Friedman & Rosbash (1977), using, however, 80–125 μL dCT^{32}P. Excellent incorporation (up to 80% of dCT^{32}P into polynucleotide) was obtained.

Reactions in (a) or (b) were stopped by addition of EDTA to 10 mM and frozen at −20 °C. The labeled product was separated from unincorporated nucleotides by chromatography on Sephadex SP C-50 in 300 mM NaCl–10 mM NaOAc (pH 5.0). The labeled product was used in this buffer or ethanol precipitated if necessary to concentrate it.

(*c*) *Flow-through (F-T) RNA.* In vitro transcription in yeast nuclei was performed and newly initiated RNA separated from nascent elongated RNA by the γ-S nucleotide, Hg–agarose chromatography technique of Reeve et al. (1977). This was performed as described in Ide (1981). Flow-through RNA is thus nascent, in vitro elongated RNA.

Results

Quantitation of Transfer Efficiency. To obtain the necessary resolution required to see structural detail in nuclease digestion patterns, I needed to use polyacrylamide gels of higher percentages than have previously been used in transfer experiments. To determine how efficiently the combination of electrophoretic transfer and DBM paper can transfer and bind DNA from these high-resolution gels, I quantitated the transfer process with ^{32}P-end-labeled PM2–*Hae*III restriction fragments. Table I shows that particularly in the size ranges of interest here (100–300 bp), there is a good transfer efficiency with this system. Transfer from urea gels always is less efficient; possibly residual urea reacts with the DBM groups

Table I: Efficiency of Transfer of Labeled DNA from High-Resolution Polyacrylamide Gels[a]

PM2-*Hae*III DNA fragment size	3.1% polyacrylamide–0.6% agarose gel (area under curve)		efficiency into DBM (%)	5.5% polyacrylamide–0.4% agarose–7 M urea gel (area under curve)		efficiency into DBM (%)	
	"dried"	"transferred"		"dried"	"transferred"		
H = 525 bp	0.0209	0.00145	7	–	–	–	H
I = 336 bp				0.0211	0.0022	10	I
J = 301 bp	0.0647	0.0379	59				J
K = 272 bp				0.0552	0.0075	14	K
L = 168 bp	0.0569	0.0528	93	0.0515	0.0166	32	L
M = 152 bp				0.0337	0.0088	26	M
N = 121 bp	–	–	–	0.0478	0.0122	25	N
O = 97 bp				0.0458	0.0083	18	O

[a] Two tracks of ^{32}P-end-labeled restriction fragments (PM2-*Hae*III) were electrophoresed on a particular composite gel. One track of fragments was cut out and Cl_3CCOOH fixed, and the gel was dried and autoradiographed ("dried"). DNA in the other track was electrophoretically transferred to DBM paper and autoradiographed ("transferred"). The films were scanned and areas under peaks cut out and weighed. Each type of composite gel quantitated is the standard one for each of the two types of nuclease digests analyzed in this paper, and in fact, in each of these cases, DNA from a nuclease digest was simultaneously transferred with the PM2 fragments.

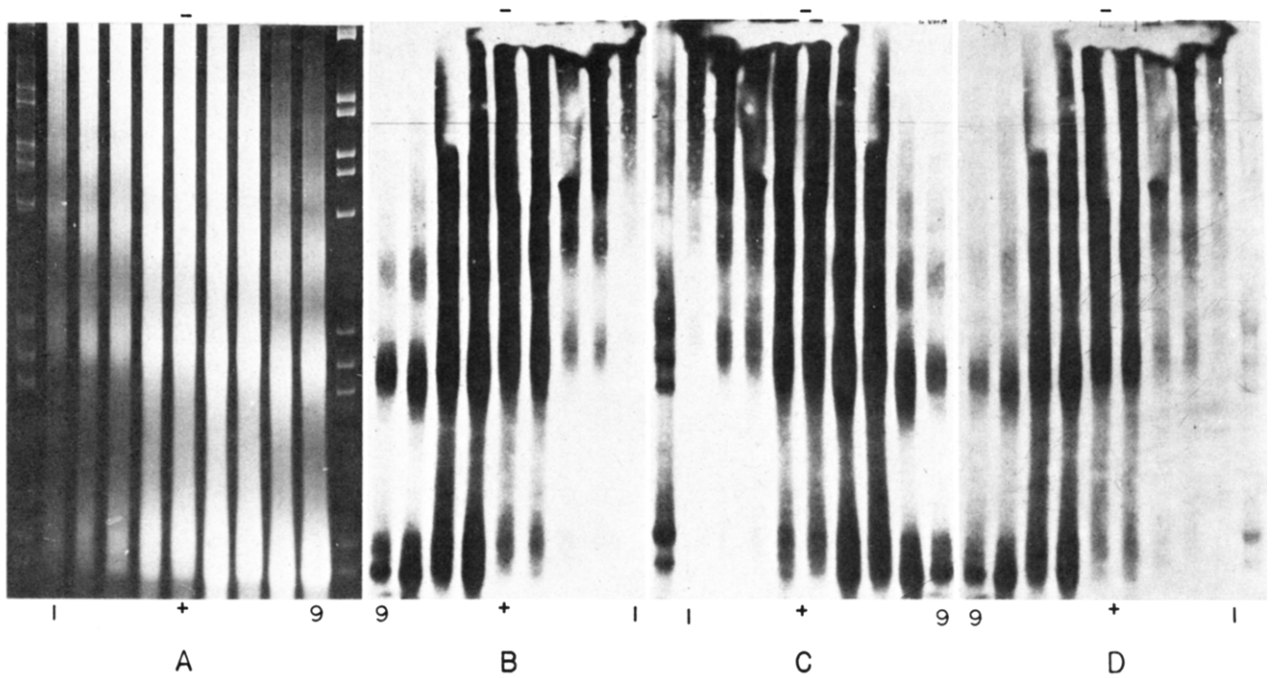

FIGURE 1: Staphylococcal nuclease digestion patterns of transcribed vs. bulk DNA. DNA from staphylococcal nuclease digestion time courses produced by digestion of nuclei from growing cells [(track 1) 90 s, (track 3) 3 min, (track 5) 8 min, (track 7) 16 min, and (track 9) 40 min of digestion] and stationary phase cells [(track 2) 90 s, (track 4) 4 min, (track 6) 12 min, and (track 8) 30 min of digestion] were electrophoresed on a 3.3% polyacrylamide/0.7% agarose composite gel, direction from top to bottom, stained with ethidium bromide and photographed (panel A). *Hae*III restriction endonuclease fragments of PM2 phage DNA, ^{32}P-labeled (track 0) and unlabeled (track 10), were used as marker fragments. DNA was electrophoretically transferred to DBM paper and probed with several ^{32}P-labeled probes in order of hybridization: (panel C) cDNA, (panel D) flow-through RNA, and (panel B) end-labeled total genome DNA. The labeled PM2 is not shown in panel B. Adjacent gels are shown in reverse to facilitate direct detailed comparison, i.e., from left to right. (A) Tracks 0–10; (B) tracks 9–1; (C) tracks 0–9; (D) tracks 9–0.

on the paper. A variable transfer efficiency does not compromise the kinds of analyses in this paper because I rehybridize the same pieces of paper with different probes. Thus all probes encounter the same DNA profile on any single sheet of paper.

Uniform Nucleosome Structure for Transcribed and Bulk DNA Sequences. (A) Staphylococcal Nuclease. Figure 1 shows the results of an experiment to analyze the detailed nucleosome structure of transcribed DNA sequences. DNA from the ethidium bromide stained gel in Figure 1A was electrophoretically transferred to a piece of DBM paper and hybridized successively with several ^{32}P-labeled probes: (1) complementary DNA synthesized against poly(A+) mRNA by reverse transcription (cDNA, Figure 1C); (2) nascent, in vitro nuclear elongated RNA (F-T RNA, Figure 1D); (3) sonicated total genomic ^{32}P-end-labeled DNA (Figure 1B). After autoradiography of the hybridization patterns from a given probe, the probe was removed from the paper as described under Experimental Procedures, and then the paper was hybridized with another probe. DBM papers after as many as six such hybridization and wash cycles show no detectable loss of efficiency of hybridization.

The immediate impression from the data shown in Figure 1 is that there is really little difference between the hybridization patterns of the transcribed DNA (cDNA or in vitro elongated RNA) and the total genomic DNA. The total pattern is shown by the ethidium bromide and the kinased total genomic hybridization. These two patterns appear very similar, as they should. The kinased pattern appears somewhat "cleaner", having a lower residual background level and

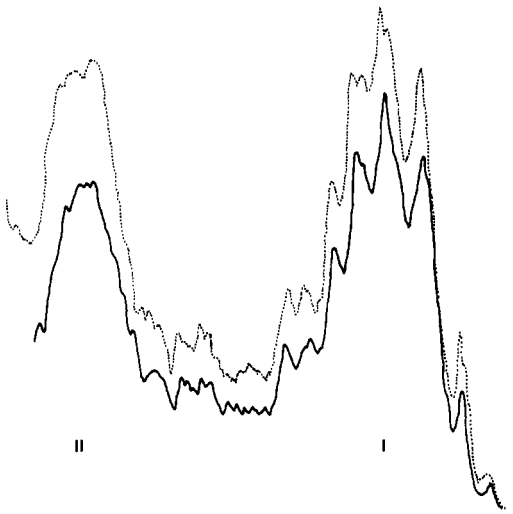

FIGURE 2: High-resolution analysis of transcribed vs. bulk dimer (II) and monomer (I) length DNA. DNA from staphylococcal nuclease digestion time courses produced by digestion of nuclei from growing cells and from stationary phase cells was electrophoresed on a 4.5% polyacrylamide/0.6% agarose composite gel, and DNA was electrophoretically transferred to DBM paper and probed with ^{32}P-labeled cDNA (---) and then with kinased total genomic DNA (—). A number of times of digestion were scanned on a densitometer. The representative scans shown here correspond to DNA seen in track 7 from Figure 1. Electrophoresis is from left to right.

sharper band resolution. I consistently note that in such comparisons of ethidium bromide and autoradiographic patterns.

The extensive similarity of the patterns obtained with the active probes and the total probe was surprising. In order to objectively analyze the data, I have scanned the autoradiograms on a Joyce-Loebel microdensitometer. The densitometric analysis confirms the similarity. There is no consistent difference in length of DNA associated with nucleosomes on the DNA sequences which code for poly(A)-containing RNA or on sequences containing nascent, elongatable RNA in yeast nuclei. Since the length of DNA, which accumulates as a transient intermediate late in digestion, the 148-bp core particle length, is also the same for nucleosomes on active sequences (cf. tracks 7–9, Figures 1C,D), the predominant lengths of spacer DNA associated with the nucleosomes on these transcribed sequences must also be the same as for the entire genome.

Careful analysis of the bands of DNA produced during staphylococcal nuclease digestion of total yeast chromatin shows the presence of various transient intermediates (Lohr et al., 1977b). Such heterogeneity within a band of protected DNA could arise from internucleosome heterogeneity or from the presence of structural features within the nucleosome which cause some transient inhibition of staphylococcal nuclease digestion. Whatever the cause, the result is a bi- or triphasic DNA peak reflecting these intermediates. Such profiles have been observed (Lohr et al., 1977b; Martin et al., 1977) and are particularly apparent in mono- and dinucleosome peaks. The scans shown in Figure 2 analyze for the presence of these intermediates in active nucleosomes and also demonstrate the precision of the method developed here; DNA was electrophoretically resolved and transferred from a 4.5% polyacrylamide gel. One can say definitively that this fine structure detail exists on nucleosomes containing transcribed DNA sequences because the substructure in the monomer and dimer DNA peaks on this high-resolution gel is virtually identical for both active and inactive nucleosomes. Thus, whatever is responsible for the production of this heterogeneity must be present in nucleosomes on transcribed and bulk DNA.

Previous observations have suggested that at least some of the DNA which is found between the main staphylococcal nuclease repeat DNA occurs as discrete lengths of DNA (D. E. Lohr, unpublished observations). This is understandable because although staphylococcal nuclease strongly prefers to hit spacer DNA, there will be *some* intracore particle hits even early in digestion. A combination of such an intracore particle cut with a spacer cut will produce DNA lengths between the main staphylococcal nuclease repeat peaks. The data in Figure 2 confirms that indeed some of these interrepeat DNA lengths are discrete and that for nucleosomes on transcribed DNA there is not any change in the frequency with which this intracore particle attack occurs, relative to the more prevalent spacer DNA attack.

(*B*) *DNase I.* Adjacent core particles in yeast pack in rather definite ways, so as to produce discrete spacer lengths of DNA (Lohr et al., 1977c) which contain a 5-bp increment (Lohr & Van Holde, 1979). This information on internucleosome arrangement is shown most clearly by DNase I because this nuclease attacks core particle DNA much more readily than staphylococcal nuclease does and thus produces significant amounts of digested DNA still containing intact spacer lengths. A discrete spacer length necessitates "correlation" in the locations of neighboring nucleosomes with respect to one another. I have termed this "phasing" (Lohr et. al., 1977c) to reflect the periodic nature of nucleosome locations. Another use of the term "phasing" refers to DNA sequence specific location of nucleosomes. In this paper, the term phasing describes correlated, adjacent nucleosome locations without regard or reference to DNA sequence features.

In a DNA pattern produced by DNase I digestion of yeast chromatin, the DNA bands smaller than 110 bp reflect intracore particle nicks by DNase I, while the discrete DNA bands larger than 115 bp reflect intercore nicks; there is a characteristic region where the intracore pattern merges with the intercore pattern, and because there is a 5 bp increment in the spacer, the two patterns are out of phase.

Data in Figure 3 show that all details of the characteristic yeast phased nucleosome arrangement must be present identically on transcribed and nontranscribed nucleosomes because none of the general features or details of DNase I patterns are either relatively diminished or relatively exaggerated in active DNA patterns. Thus discrete nucleosome phasing must be a global, general structural feature of all yeast chromatin, even active chromatin.

To be certain that no trivial explanation could be responsible for the identity of transcribed and nontranscribed patterns, I always used an active probe (cDNA) as the first probe to hybridize to a DBM paper. Thus there is absolutely no possibility for contamination of the active pattern by any residual probe from a previous hybridization. To ensure noninterference by the active pattern with subsequent patterns, the success of washing off the previous probe was followed with the hand monitor, and rehybridization was only performed when the background level on the paper was acceptable. In addition, occasionally papers were put away and rehybridized later to ensure that not even low levels of previous probe could contribute to the pattern. For example, the kinased total genome DNA of Figure 1B was hybridized 2.5 months ($\sim$5 half-lives of ^{32}P) after the previous probe was removed from it.

Furthermore, since each probe was hybridized to the exact same DNA profile on the exact same piece of paper, one can make the most direct comparison possible and need not worry

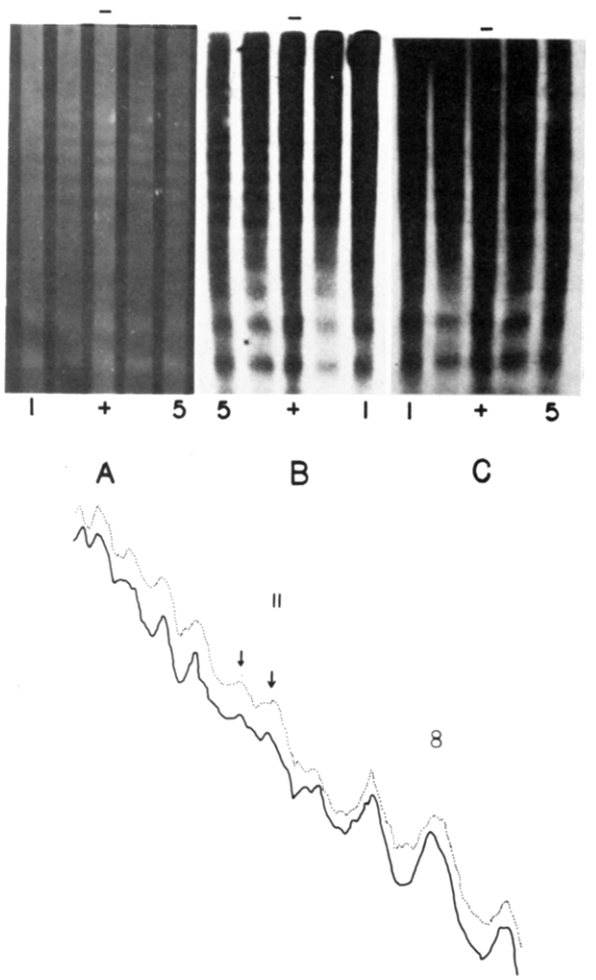

FIGURE 3: DNase I digestion patterns of transcribed vs. bulk DNA. DNA from DNase I digestion time courses produced by digestion of nuclei from growing cells [(track 1) 20 s, (track 3) 90 s, and (track 5) 3 min of digestion] and stationary phase cells [(track 2) 20 s and (track 4) 3 min of digestion] was electrophoresed on a 5.6% polyacrylamide/0.4% agarose/7 M urea composite gel, direction from top to bottom, stained with ethidium bromide and photographed (panel A). DNA was electrophoretically transferred to DBM paper and probed with ^{32}P-labeled cDNA (panel C) and then end-labeled total genome DNA (panel B). Adjacent gels are shown in reverse to facilitate direct comparison, i.e., from left to right, (A) tracks 1–5, (B) tracks 5–1 and (C) tracks 1–5. For a clear illustration of the exact coincidence of patterns, scans of panel B (track 1) [total genomic DNA pattern, (—)] and panel C (track 1) [cDNA pattern (···)] are compared in (D). DNA bands 8 (84 nucleotides) and 11 (115 nucleotides) are marked. The double arrows point to the two closely spaced bands which delineate the region where the DNA pattern from intracore and intercore nicks overlaps. This transition region is diagnostic for phasing.

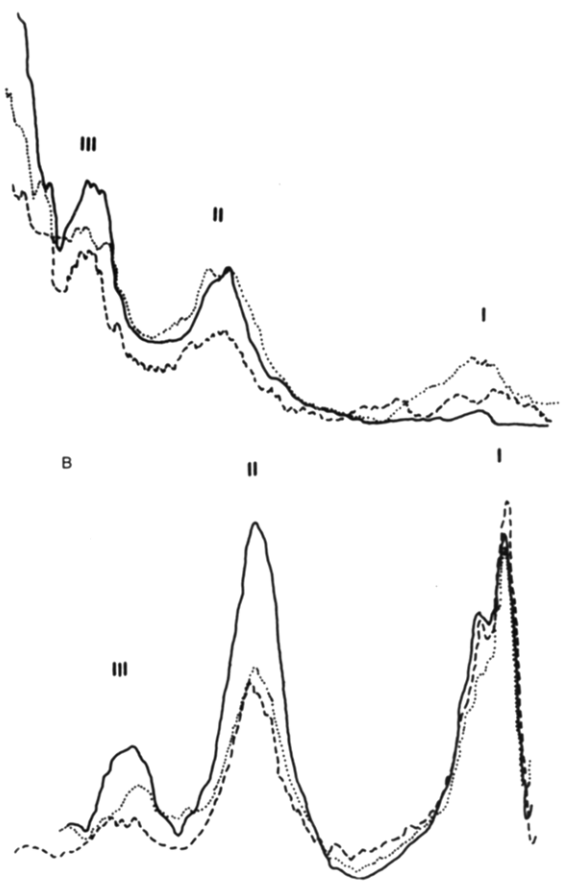

FIGURE 4: Representative scans from staphylococcal nuclease time courses of digestion comparing patterns of transcribed vs. bulk DNA. Autoradiographic patterns obtained by using cDNA (···), flow-through RNA (- - -), and total genomic DNA (—) as probes were scanned on a Joyce-Loebel microdensitometer. (A) Scans corresponding to track 1 (90 s of digestion) of Figure 1 showing monomer (I), dimer (II), and trimer (III) DNA peaks. (B) Scans corresponding to track 9 (40 min of digestion) of Figure 1, again show trimer (III), dimer (II), and monomer (I) DNA. Electrophoresis is from left to right in each case.

about variations in reproducibility involved in comparison of transfers from different gels. Using a radiolabeled, total sequence probe rather than relying on the ethidium bromide profile to obtain the bulk pattern automatically compensates for the size dependence of the transfer and hybridization efficiency within a gel track. Thus it is not necessary to attempt to correct for this variation either.

Unique Features of Active Nucleosomes. Although the previous data convey the overwhelming impression that transcribed nucleosomes resemble the bulk ones, careful densitometry shows that there are some subtle distinctions, at least early in staphylococcal nuclease digestion.

First, in general, yeast shows variation in repeat lengths within its genome. However, early in staphylococcal nuclease digestion the active nucleosomes seem to contain an even broader distribution of repeat lengths, which can be analyzed particularly conveniently in the dimer DNA peak (Figure 4). Peak widths at 0.5 or 0.75 of maximum peak height reflect the intrinsic heterogeneity of DNA sizes in the peak. For 90 s of digestion (Figure 4A), the widths are 35 bp for total kinased DNA as probe against 53 and 57 bp for cDNA and flow-through RNA as probe. At 40 min of digestion (Figure 4B), the widths are 30 bp for all three probe patterns. Thus active dimer peaks show significantly greater heterogeneity of sizes but only early in digestion.

Another differential feature noted is an increased level of monomer DNA relative to oligomer DNA in the active sequence patterns. Because these gels must be very high resolution in order to see structural details, it has not been possible to analyze the total profile, including larger DNA. Thus I have compared the intensity of monomer DNA relative to dimer, trimer, and tetramer levels in active and inactive nucleosomes. Early in digestion, patterns which have been probed with active probes show significantly higher levels of monomer length DNA. This tendency is also apparent late in digestion (Figure 4B). Because the total sequence probe is end-labeled and hence every size of probe has only one ^{32}P while the active probes are uniformly labeled, the difference between active and total is even more pronounced than it appears because total probe will underrepresent larger DNA compared to its representation by active probes. These observations are consistent with an accelerated rate of core particle release by staphylococcal nuclease digestion of the active nucleosomes.

Stationary vs. Growing Cells. Chromatin digests using nuclei isolated from stationary cells have been analyzed in parallel with the digests of chromatin from growing cells on all the gels analyzed during these experiments. The stationary profiles were probed with cDNA or in vitro elongated RNA from growing cells or kinased total genome DNA.

Just as for the profiles from growing cells, the staphylococcal nuclease and DNase I digest profiles from stationary cells probed with the various active and total probes all look basically identical. Careful densitometry shows that some of the subtle differences noted in digests from growing cells remain. For example, the tendency for preferential accumulation of monomer DNA in digestion is still quite apparent in stationary digests, but the increased peak breadths noted with active probes of growing phase DNA profiles are not as prominent. This structural data for stationary phase chromatin is consistent with previous data, suggesting that the transition to stationary phase is readily reversible and therefore probably involves little, if any, structural rearrangement (Lohr & Ide, 1979), although there is a profound alteration in transcriptional activity.

Discussion

Perhaps surprisingly, the data in this paper show that structurally, nucleosomes containing transcribed DNA in yeast closely resemble the average, typical, bulk yeast nucleosome. Nucleosomal and core particle DNA lengths, various measures of heterogeneity within (and between) the given DNA peaks, noted in staphylococcal nuclease digests, and phasing of adjacent nucleosomes with inclusion of a 5-bp increment in the spacer, noted by DNase I digestions, all appear to be equally characteristic of transcribed and bulk DNA. Furthermore, the strong parallelism of patterns through entire time courses of digestion suggests that there is little difference that staphylococcal nuclease or DNase I can recognize between transcribed and total sequence nucleosomes in yeast.

It would be useful to know the generality of this conclusion among eukaryotic chromatins. Unfortunately, sufficient detailed structural data are not available. However, there are some clear qualitative differences. For example, in the most structurally detailed analyses of nonribosomal genes in multicellular eukaryotes, Wu et al. (1979) and Levy & Noll (1981) find that *active* heat shock genes do not show a typical nucleosome repeat pattern. On the other hand, there is evidence that other active genes, especially ovalbumin, do show at least aspects of the typical nucleosome repeat pattern (reviewed in Mathis et al., 1980). Structural analysis of DNase I patterns of active genes is not available. However, there is general agreement that active genes in multicellular eukaryotes show preferential, kinetic DNase I sensitivity. On the other hand, earlier work in yeast showed that this most definitive distinction of active DNA in multicellular eukaryotes is not a characteristic of transcribed yeast DNA (Lohr & Hereford, 1979). This was interpreted to mean that the entire yeast genome behaves as if it were active chromatin, at least by the criterion of preferential, kinetic DNase I sensitivity. Thus intragenome comparison of DNase I sensitivity of transcribed to total sequences shows no differences.

Such a model, in which all the yeast chromatin behaves like activated or transcribed chromatin, would readily explain why I observe so little difference in structural detail between transcribed and total yeast nucleosomes, as shown in this paper. There is simply not enough (or any) intragenomic, inactive chromatin with which to contrast the active sequences. Obviously a uniformly activated genome does not generally characterize multicellular eukaryotes. But, in yeast, since more than 40% of the genome is transcribed into (mature) poly(A)-containing mRNA under normal conditions of growth (Hereford & Rosbash, 1977), merely doubling the extent of the activated, susceptible conformation would produce a uniformly activated genome. For example, Bellard et al., (1980) have shown that the activated state, as reflected by DNase I sensitivity, extends several hundred nucleotides beyond the 5′ end of the ovalbumin gene in chicken. If such is a general situation, a 2-fold extension of the activated state in yeast to include the other ~50% of the DNA not detectably transcribed seems quite reasonable and thus provides a plausible explanation of the structural digestion data.

On the other hand, one could argue that there were no more striking chromatin structural differences noted here between transcribed and total sequence nucleosomes because neither of the active probes used adequately reflects active sequences. For example, although flow-through RNA was clearly being transcribed in nuclei, one does not know whether *all* the nuclei present were transcribing *all* the sequences represented in that RNA population. One could argue for cDNA that at least some of the RNA may have been made at an earlier time and stored (Mathis et al., 1980) and thus did not really reflect gene activity. In defense, one should point out that the very rapid cell cycle time of yeast makes large-scale RNA storage unlikely, only polysomal poly(A+) mRNA, i.e., RNA actually being translated, was used to make cDNA, and use of cDNA as an active gene probe *can* be successful for it has been shown in trout (Levy W. & Dixon, 1977), with a longer cell cycle time, that DNA sequences coding for poly(A+) mRNA reflect the characteristic DNase I kinetic differences seen in single gene studies of active sequences. Furthermore, in yeast, the poly(A+) mRNA population is dominated by a small number of sequences [~25% consists of about 20 sequences present in around 200 or so copies per cell and ~50% consists of about 400 sequences present in about 20 copies per cell (Hereford & Rosbash (1977)] so that cDNA made from this mRNA mainly probes the structure of this limited set of genes which would be expected to be transcribed at the maximal rate for a yeast nonribosomal gene (since this mRNA is present in highest concentration). However, total repudiation of this type of objection is probably impossible for any work in this area, even for single gene analyses, for it is difficult to be entirely sure that all cells in a population or even all genes in a genome (since most organisms are diploid) are expressing the gene of interest.

The subtle differences noted (greater monomer concentration and greater peak breadths) in this work suggest that there is a subset of transcribed sequences in yeast which have somewhat altered properties. Recently, Lamb & Daneholt (1979) observed that the active 75S transcription unit in *Chironumus* contains nucleosomes which pack less densely and less regularly than nucleosomes on the inactive chromatin. Furthermore, they point out that the nucleosome packing density (beads/μm) decreases with increasing RNA polymerase loading in a number of situations, suggesting that this decreased packing density in transcribing chromatin may result from recent polymerase passage. Clearly there must be longer spacer lengths of DNA between these less densely packed, active nucleosomes. Since the primary target of staphylococcal nuclease is the spacer, these more infrequently spaced nucleosomes would present a preferred target for staphylococcal nuclease and should be preferentially digested to contribute to a population of more quickly released mononucleosomes. Oligomeric active DNA would show a broader distribution of lengths by the inclusion of such longer, irregular spacers. In

fact, the increase in oligomer peak breadth noted in this work occurs mainly on the high molecular weight side of the DNA peak as would be expected from such an explanation. Thus the behavior of the subset of active sequences I note is entirely consistent with expected digestion properties of the kinds of structures seen by Lamb & Daneholt (1979). If these occur mainly in the vicinity of *transcribing* RNA polymerase, one can understand why these structures constitute only a subset of the totality of *transcribed* sequences. At least some aspects of this type of distinction are also found in other eukaryotes, for one can obtain preferential production of mononucleosome DNA from active chromatin regions (Bellard et al., 1978). Thus, even though yeast shows no distinction of transcribed chromatin from bulk chromatin (reflected in the uniform genome DNase I sensitivity), there seems to be some distinction of transcribing chromatin from nontranscribing.

This interpretation would suggest that within the yeast genome, the main alterations of nucleosome structure in active regions are changes involving spacer DNA. However, the lack of any large scale structural change in transcribed yeast nucleosomes would seem to preclude any extensive, long-lived or long-ranged alteration unless the alteration has no effect on nuclease digestion characteristics. Thus these results suggest that at least in yeast, the presence of nucleosomes may restrict transcription less than is commonly envisioned, DNA in yeast nucleosomes being transcribable as is or after some transient, readily reversible alteration in structure. The observation that growing phase and stationary chromatin are structurally quite similar but differ profoundly in in vitro and in vivo transcriptional activity (Lohr & Ide, 1979) support the conclusion that extensive transcriptional activity changes can occur without (detectable) nucleosome structural alterations.

In most known details (except in size and arrangement of spacer DNA, interestingly), yeast nucleosome structure resembles multicellular eukaryotic nucleosome structure (Lohr et al., 1977a,b). Thus intrinsic transcribability may be a more general feature of nucleosomes. In vitro transcription data suggest a similar conclusion (Green & Brooks, 1977; Williamson & Felsenfeld, 1978; Wasylyk & Chambon, 1979).

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