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The chromatin structure of an actively expressed, single copy yeast gene

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ABSTRACT

When the yeast galactokinase gene is not active (repressed, not expressed, quiescent), there is an exceptionally regular nucleosome array on coding sequence galactokinase chromatin, as shown by both denaturing and non-denaturing gel analysis of staphylococcal nuclease digests. Expression of the gene results in a limited smearing of the nucleosome repeat peaks and an increase in interpeak DNA, appearing as a regular ladder of DNA bands on denaturing gels. On non-denaturing gels the pattern is more complex and molecular weight dependent. These data suggest an increase in intracore particle DNA accessibility, allowing staphylococcal nuclease to digest throughout the nucleosome in expressed chromatin. Comparison to bulk chromatin and to an operationally inactive gene (35S rDNA) show that the alteration is specific to expressed chromatin. In contrast, DNase I shows no differences in the digestion of the gene specific chromatin in expressed or inactive states.

INTRODUCTION

In vivo, eukaryotic DNA exists as a nucleoprotein complex. The most basic unit of this complex is the nucleosome, a topologically constrained structure in which the DNA wraps around a histone octamer, probably in a left-handed (toroidally supercoiled) orientation. Continued association in a nucleosome would seem likely to cause problems during transcription of DNA. Accordingly, most transcription models have postulated removal of histones from the site of transcription by sliding along the DNA, transfer to one strand or complete dissociation.

Indeed, it has been shown that the nucleosome repeat pattern produced by staphylococcal nuclease disappears from Drosophila heat shock chromatin when the genes are expressed (1,2). However, it is possible that histones remain on the active chromatin but simply rearrange to a structure which does not yield the usual repeat pattern. This is consistent with the reversibility of the altered pattern (1,2), with earlier biochemical data which suggests subtle alterations in the structure of "active" chromatin

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(reviewed 3) and with suggestions from RNA synthetic experiments that transcription can occur without histone dissociation (5-8). Also, Moyne et al. (4) have shown a nucleosome rearrangement in SV40 minichromosomes.

The heat shock gene studies suggest that the most definitive results will be obtained by studying the chromatin structure of highly transcribed genes which are being maximally expressed. One can insure that all copies of the gene being studied are expressed by analyzing a selectable gene in haploid cells. Yeast galactokinase, a highly expressed gene which codes for one of the enzymatic activities needed for utilization of galactose as a carbon source, fulfills the above requirements. This work describes the analysis of the chromatin structure on this gene as a function of expression state.

MATERIALS AND METHODS

Cells were grown to early log (5 x 10^7 per ml) or stationary (20-30 x 10^7 per ml) in YEPD, YEPG or YEPE (1 percent yeast extract, 2 percent Bactopeptone, 2 percent dextrose, galactose or ethanol). Nuclei were isolated and digestions performed with staphylococcal nuclease or DNase I and DNA extracted as described (9). DNA was electrophoresed on composite non-denaturing or denaturing gels (4-5.5 percent polyacrylamide/0.4% Agarose in 7 M urea) and transferred to DBM paper exactly as described in (10). Hybridizations were done according to (11).

The plasmid Sc4817 was grown in L broth + tetracycline, chloramphenicol amplified and isolated by gentle lysis followed by CsCl/ethidium bromide equilibrium gradient centrifugation as described in (12). The yeast insert was removed by Eco RI and Pvu II restriction, followed by isolation of the two fragments, an RI - Pvu II fragment, ≈ 850 bp and a Pvu II - RI fragment, ≈ 250 bp, on a Savant preparative electrophoresis cell.

Hybridization probes were made from this DNA by: 1) repairing ends of restriction fragments with overlapping termini (Eco RI, Sau3A) using [α^{32}-P] dTTP and [α-^{32}P] dATP as described in (13) plus cold dGTP for the Sau 3A ends; 2) by labelling 5' -OH ends of restriction fragments (treated with alkaline phosphatase) with [γ-^{32}P]ATP and T_4 polynucleotide kinase. After labelling, single strand probes were obtained by restriction digestion. Labelled fragments were isolated on 5.5 -7% polyacrylamide gels by the methods described in (14). Probes with one labelled strand were tested by redigesting with other restriction enzymes with known cleavages (Citron and Donelson, private communication) to produce positive results i.e., a single strand probe should yield only 1 smaller piece of DNA on redigestion. Double strand probes were obtained by repairing a Sau 3A digest of the yeast insert followed by gel isolation of the individual fragments. It was possible to get good yields of Sau3A a+b (≅ 190 bp), because the Sau 3A site between a and b is cleaved more slowly than others. Autoradiography was done with X-ray film XAR-5 at -80°C using Dupont Cronex intensifying screens.

RESULTS

The expression state of the galactokinase gene depends on the carbon

source. In cells grown to early log phase in galactose, the gene is expressed. Because the cells are growing most rapidly, the gene should be expressing maximally. For comparison, cells can be grown to early log phase in glucose, where the gene is repressed to zero level of expression (15,16) or to early log phase in ethanol, where the gene is no longer repressed but is still not expressed. In carbon sources like ethanol the gene can be quickly (a few minutes) induced to activity and thus can be considered to be in a state between repressed and expressed, an "expressible" state.

To obtain chromatin structure data, DNA isolated from staphylococcal nuclease and DNase I digestions of nuclear chromatin from these various growth states was electrophoresed under denaturing conditions (and non-denaturing conditions for staphylococcal nuclease analysis), transferred to DBM papers and hybridized with probes from various parts of the galactokinase gene. Figure 1 shows a restriction map of the gene.

Staphylococcal Nuclease Digestion Patterns.

Inactive Chromatin. When the gene is not being expressed, i.e., in ethanol or glucose grown cells, staphylococcal nuclease digestion of galactokinase chromatin produces a clear nucleosome ladder, whether visualized on

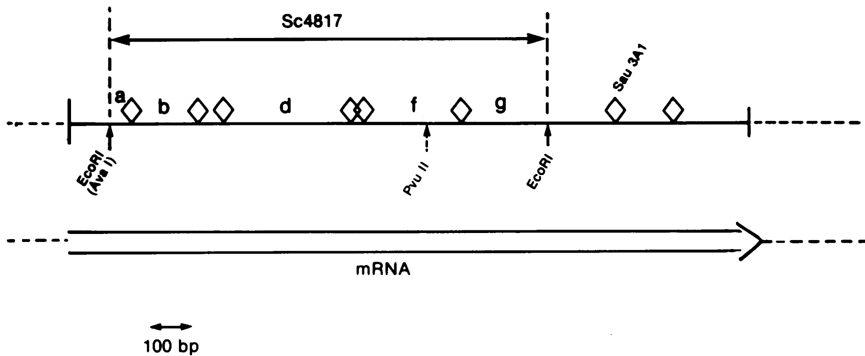

Figure 1. Restriction Map of the Yeast Galactokinase Gene

The ~ 1580 bp of coding sequence of the galactokinase gene is shown (solid line) with some pertinent restriction enzyme sites. Various parts of the ~ 1100 bp of DNA from the Ava I site to the Eco RI site, contained on the recombinant plasmid Sc4817 (17) were used as probes. In this plasmid, the chromosomal Ava I site has been changed into an Eco RI site (St. John, private communication) so that the plasmid contains Eco RI sites on each end. The Sau 3A sites are shown as ◇. Letters a - g refer to the various Sau3A fragments obtainable from a digest of this plasmid. The direction of transcription is shown below the gene. The arrow indicates nothing concerning in vivo initiation and termination sites of the RNA, but the vertical line on the left end of the gene locates the translational initiation codon.

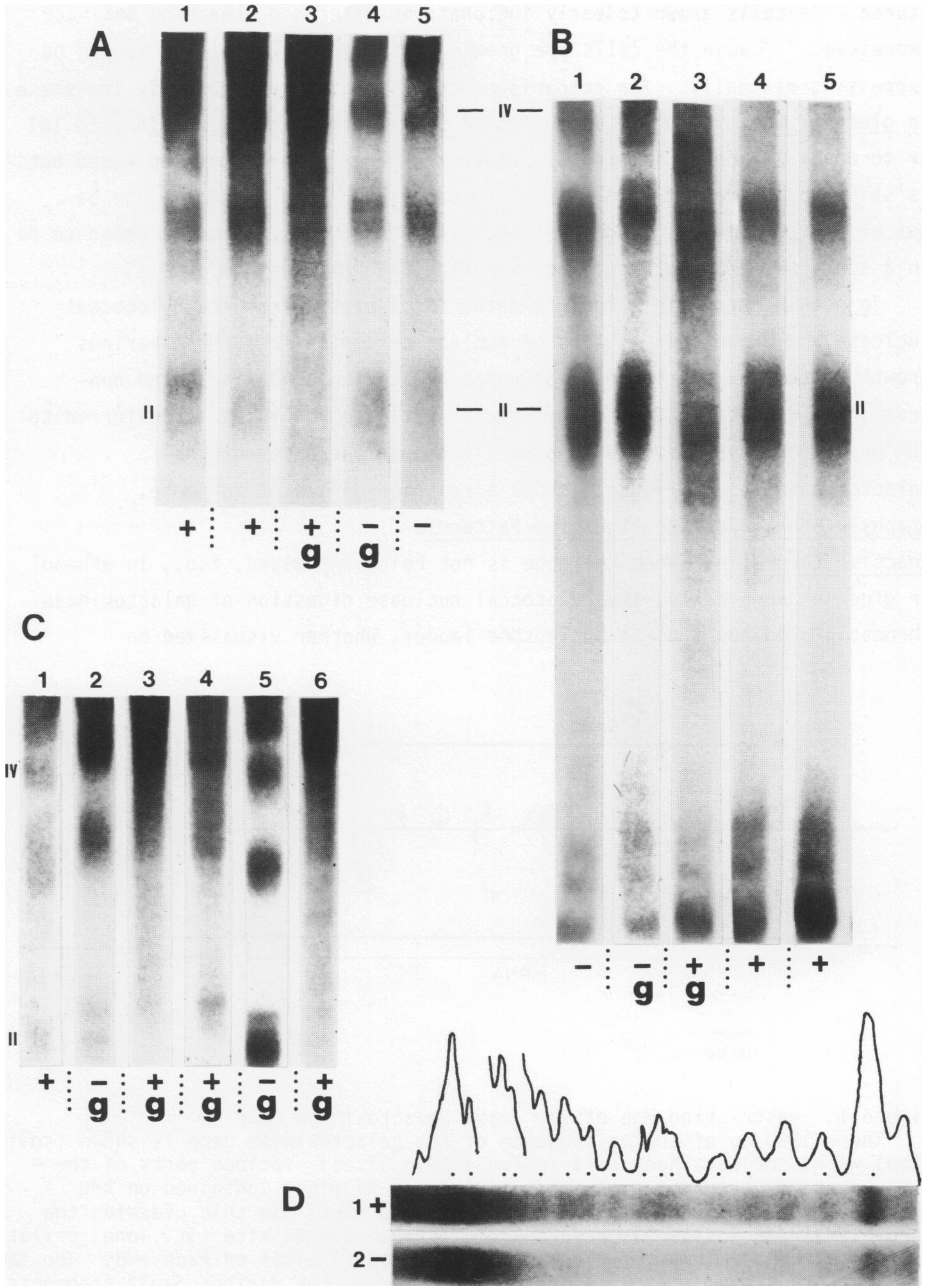
A
1
2
3
4
5
II
+
+
+
g
–
g
–
B
1
2
3
4
5
— IV —
— II —
II
–
–
g
+
g
+
+
C
1
2
3
4
5
6
IV
II
+
–
g
+
g
+
g
–
g
+
g
D
1 +
2 –

Figure 2. Staphylococcal Nuclease Digestion Patterns of the Galactokinase Gene in Various States of Expression (Denaturing)

DNA from staphylococcal nuclease digests of nuclear chromatin from cells grown in various carbon sources was electrophoretically separated, transferred to DBM paper and hybridized with probes from the yeast galactokinase gene coding sequences, the 35S rDNA coding sequences or bulk DNA. The state of galactokinase gene activity in the cells from which the chromatin was obtained will be indicated immediately below each gel track by "+", the gene is expressed (log phase cells in galactose) or "-", the gene is repressed (log phase in glucose), not expressed (log phase in ethanol) or quiescent (stationary phase in galactose). The second row below the track indicates those tracks where a galactokinase specific probe was used ("g", to visualize galactokinase chromatin). Thus "+" tracks, with and without "g", compare the chromatin structure of different genes (galactokinase or rDNA) or bulk DNA, within the same cell. These profiles were obtained by rehybridizations of the same DBM track (after previous probe removal) with the appropriate probes. The "+g" and "-g" tracks compare the galactokinase chromatin in various states of gene activity.

The various autoradiograms were lined up with one another by including end-labelled PM2/Hae III DNA fragments in each hybridization, to visualize the PM2 - Hae III fragments present. The Roman numerals II and IV locate the positions of di- and tetranucleosomal DNA. Electrophoresis is from top to bottom, except in D where it is from left to right.

Gel	Track	Growth State/ Carbon Source	Galactokinase Gene	Probe
A)	1	log / galactose	+	35S rDNA
	2	" "	+	bulk DNA
	3	" "	+	RI-Pvu II (850 bp)
	4	log / ethanol	-	"
	5	" "	-	bulk DNA
B)	(more extensive digestion, same gel as A)			
	1	stationary / galactose	-	bulk DNA
	2	" "	-	RI-Pvu II (850 bp)
	3	log / galactose	+	"
	4	" "	+	bulk DNA
	5	" "	+	35S rDNA
C)	1	log / galactose	+	bulk DNA
	2	" / glucose	-	Pvu II-RI (250 bp)
	3	" / galactose	+	"
	4	" "	+	"
	5	" / glucose	-	"
	6	" / galactose	+	Sau3A a+b

D) The di- to mononucleosomal region of similar chromatin digests from log (+) and stationary phase (-) galactose grown cells was probed with the 250 bp Pvu II - RI fragment from the galactokinase gene. The active and inactive profiles were matched for intensity in the dinucleosome region. A densitometer scan of track 1 is shown immediately above the panel. Bands are denoted by "•". The second band from the right in the densitometer scan is ~ 168 nucleotides while the second band from the left is ~ 336 nucleotides.

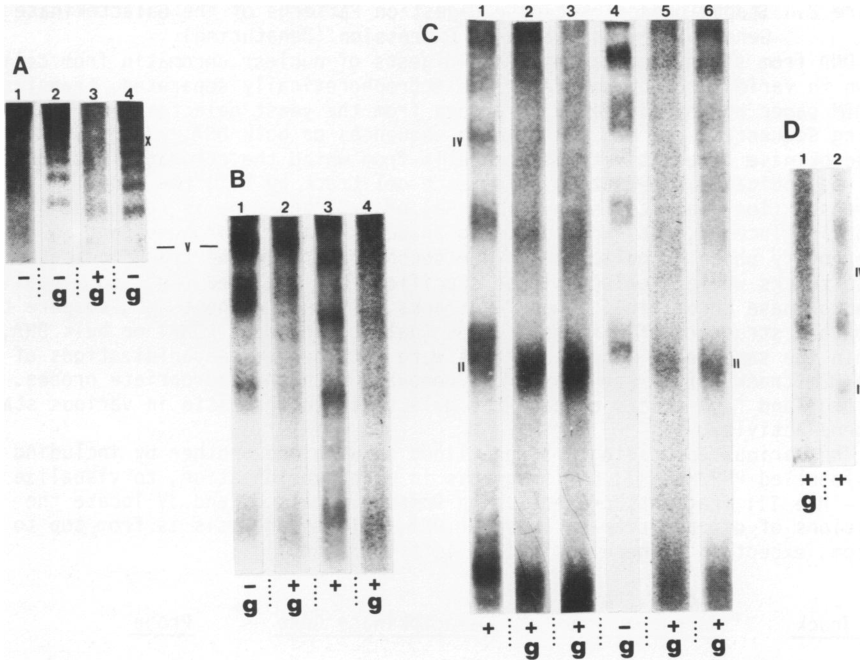

Figure 3. Staphylococcal Nuclease Digestion Patterns of the Galactokinase Gene in Various States of Expression (Non-Denaturing)

DNA from staphylococcal nuclease digests of nuclear chromatin from cells grown in various carbon sources was electrophoretically separated, transferred to DBM paper and hybridized with various probes from the galactokinase gene coding sequences, 35S rDNA coding sequences or bulk DNA. The state of galactokinase gene activity in the cells from which the chromatin was obtained will be indicated immediately below each gel track by "+", the gene is expressed (log phase galactose cells) or "-", the gene is repressed (log phase in glucose) or quiescent (stationary phase in galactose). The second row below the track indicates whether a galactokinase specific probe was used ("g"). Thus "+" tracks, with and without "g", compare the chromatin structure of different genes (35S rDNA or galactokinase) within the same cell. These profiles were obtained by rehybridization of the same DBM track with the appropriate probes. The "+g" and "-g" tracks compare the galactokinase gene itself in various states of gene activity.

Autoradiograms were lined up by including ^{32}P end-labelled PM2/Hae III restriction fragments in each hybridization, to visualize the PM2/Hae III DNA fragments present. The Roman numerals II, IV, V and X locate the positions of the di-, tetra-, penta- and decanucleosomal DNA. Electrophoresis is from top to bottom.

Gel Track	Growth State/ Carbon Source	Galactokinase Gene	Probe
A) (very light exposure to visualize oligomers)			
1	log / glucose	-	bulk DNA
2	" "	-	Sau3A a+b
3	log / galactose	+	"
4	stationary / "	-	"

B)	(lower molecular weight region, same gel as A)			
	1	stationary / galactose	-	Sau3A a+b
	2	log / "	+	"
	3	" "	+	35S rDNA
	4	" "	+	RI-Pvu II (850 bp)
C)	(a more extensive digest)			
	1	log / galactose	+	35S rDNA
	2	" "	+	Pvu II-RI (250 bp)
	3	" "	+	"
	4	" / glucose	-	"
	5	" / galactose	+	Sau3A a+b (coding) strand)
	6	" "	+	Sau3A a+b (non-coding strand)
D)	(agarose gel)			
	1	log / galactose	+	RI-Pvu II (850 bp)
	2	" "	+	bulk DNA

denaturing (Figures 2A tr 4, 2C trs. 2,5) or non-denaturing (Figures 3A, tr 2, 3C tr 4) gels. It is possible to resolve many more nucleosome peaks (up to 12-14) than can ever be resolved in bulk chromatin digests, even in brief exposures (Figure 3A, tr 1). The bulk chromatin resolution level on the autoradiograms is consistent with that seen when total DNA is visualized by ethidium bromide staining.

The increased clarity in the inactive galactokinase chromatin profile could reflect simply a narrower distribution of nucleosome repeat lengths in this chromatin. At some number of nucleosome repeats, the accumulated differences resulting from the mixture of repeat lengths in the population become significant with respect to the size difference between nucleosome peaks. This limits the resolution. The narrower the distribution of repeat lengths, the larger the number of repeats required to achieve this significant accumulation and hence the better the resolution. There may also be DNA sequence specific positioning of nucleosomes on the inactive gene (experiments are in progress). However, since precise positioning also results in a narrower repeat length distribution, it is clear that, at least, adjacent nucleosomes in inactive galactokinase chromatin are located more regularly with respect to each another than nucleosomes in bulk chromatin. A regular nucleosome profile may be a general characteristic of inactive chromatin (cf. 18,19).

Increased resolution in the inactive gene profile compared to bulk also results from the general high level of expression in yeast. Since almost half of the yeast genome is transcribed sometime during the cell cycle (20),

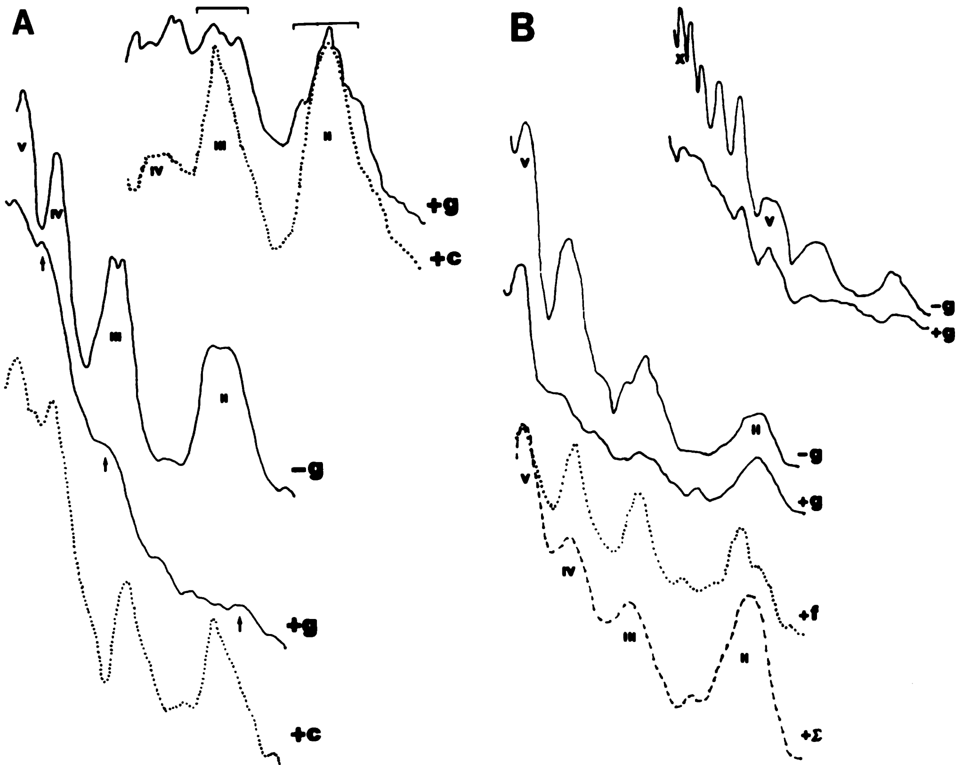

Figure 4. Densitometer Traces of Autoradiograms of Denaturing and Non-Denaturing Gel Profiles of Staphylococcal Nuclease Digestion of Chromatin.

A) Scans of the denaturing gel profiles of track 1 (+c), track 3 (+g) and track 4 (-g) of Figure 2A are shown. Some of the residual peaks at the nucleosome repeat positions in the expressed gene profile are shown ("↑"). In the upper right are scans corresponding to panel 3 (+g) and panel 5 (+c) of Figure 2B. The increased breadth of nucleosome peaks is shown by the brackets ("————") and the high background level between peaks can be seen. At no time of digestion does the profile from active galactokinase chromatin appear as clear as that from inactive chromatin.

B) Scans of non-denaturing gel profiles of track 2 (+g) and track 3 (+f) of Figure 3B are shown. The tracks corresponding to bulk DNA (+Σ) and a log/glucose profile (-g) scans are not shown in Figure 3B. In the upper right are scans which correspond to track 3 (+g) and track 2 (-g) of Figure 3A. Roman numerals II, III, IV, V and X locate the positions of di-, tri-, tetra-, penta- and decanucleosomal length DNA.

there is probably enough transcribing at any time to contribute perceptible smear to the bulk profile, if the behavior of expressed galactokinase (see below) and heat shock genes (1,2) are typical. There is no such contribution to the inactive galactokinase chromatin profile.

Active Chromatin (Denaturing). When the gene is expressed (cells actively growing in galactose), there is a disruption of the regular nucleosome pattern which is present on inactive galactokinase chromatin (Figures 2A tr 3, 2B tr 3, 2C trs. 3,4,6; 4A). There is still accumulation of DNA at the usual nucleosome repeat positions (Figures 2A, tr 3; 2B tr. 3; 4A, "↓"), but the relative amount of DNA between the peaks greatly increases. The residual nucleosome peaks also appear broadened (or "smeared") compared to the corresponding peaks in the inactive profile (c.f. Figures 2B trs. 2 vs 3; 4A "+g" vs "-g").

The interpeak DNA can become quite a significant fraction of the total (Figures 2D, 4A). In contrast, there is little detectable interpeak intensity when the gene is inactive, either early (Figure 2A, tr. 4) or later (Figure 2B, tr. 2; Figure 2C, tr. 5) in digestion, even when the intensities of the di- or trinucleosome peaks in the inactive profile are higher than the intensities in the corresponding residual active peaks. More importantly, densitometer scans (Figure 4A), which give a quantitative measure of peak height to background, clearly show the higher interpeak intensity levels in these active chromatin digests (Figure 4A, -g vs +g).

Interpeak DNA in the expressed chromatin profile can be resolved into discrete bands (Figure 2D). On this higher resolution gel, one can count 14-18 bands in the interval from 168 to 336 nucleotides (Figure 2D). Sixteen bands would give an average spacing of 10.5 nucleotides between bands, the same average spacing as the bands produced by DNase I cleavage within the core particle in yeast (21). However, this interpeak band pattern is not totally analogous to a DNase I pattern for only some of the bands in this pattern show regular spacing. The irregularities seem to occur in clusters, mainly near the center of the interpeak profile. At any rate, although there is some uncertainty in the exact number and some irregularities in the spacing, it is clear that the DNA between mono- and dinucleosome peaks is present in a specific band pattern, rather than a random smear of DNA. There are suggestions of similar regularity in the interpeak DNA between the larger residual nucleosome peaks but their resolution is beyond the resolving ability of the gels.

The structural disruption of galactokinase chromatin disappears when the cells are allowed to grow into stationary phase in galactose (Figures 2B tr 2; 2D tr 2). The clarity of the nucleosome pattern is equal to that of either non-expressed state (ethanol or glucose), which suggests that the altered pattern is associated with expression itself and not merely with a

history of, or a potential to, express. Also, as in the heat shock genes (1,2), the structural disruption of active chromatin on the galactokinase gene must be quite reversible.

Different parts of the gene [e.g. Sau 3A a+b, d, f, RI-Pvu II (850 bp) and Pvu II-RI (250 bp), some of which are shown in Figure 2] give the same results, very clear nucleosome patterns for inactive chromatin and blurring of the pattern when the gene is expressed. In addition, because of the way they were made, the Sau 3A a+b and Pvu II-RI probes detect signals from opposite strands. The similarity in patterns with these two probes suggests that the chromatin structure on the coding and non-coding strands is similar. This was confirmed by direct analysis of the patterns on each of the two strands of the Sau 3A a+b chromatin region (and of the Pvu II-RI region). For example, all probes show the particular band pattern between the mono- and dinucleosome peaks. Since DNA in this size range is approximately equal to the probe length, the majority of the hybridization signal comes from within or very near to the particular region being analyzed and thus one can know that each of the various regions and strands has been individually assessed.

The nucleosome pattern in the expressed gene profile is more smeared than the bulk chromatin pattern from the same DBM track (cf. Figure 2B, trs. 3 vs 4), which is in contrast to the clearer nucleosome profiles on inactive gene chromatin versus bulk. However, because of the high level of expression in the bulk yeast genome (20), comparisons of specific gene profiles, such as active/inactive galactokinase comparison above, are more useful. In this case, there is a possibility of artifactual differences since chromatin digests from different cells must be run on different gel tracks. To preclude this possibility, the same DBM tracks were hybridized with a 35S rDNA probe. Although some of the ~ 100 rDNA repeats must be expressed in these cells, in a nuclease digestion the gene behaves operationally like inactive chromatin because the signal is dominated by the majority, the inactive state (10). As shown in Figure 2, the nucleosome patterns in 35S digestion profiles do clearly differ from the profiles of active galactokinase chromatin (Figures 2A, trs. 1 vs. 3; 2B, trs. 3 vs. 5; 4A).

Active Chromatin (Nondenaturing). On nondenaturing gels, digests of expressed galactokinase chromatin show smeared nucleosome profiles. Both coding and noncoding strands give the same result (Figure 3C, trs. 5,6) on various parts of the gene (i.e. Sau 3A a+b, RI-Pvu II and Pvu II-RI). The

expressed profiles differ markedly from the regular nucleosome pattern seen for inactive galactokinase chromatin digests on these same gels (Figures 3A, trs. 2,4; 3B tr. 1; 3C, tr. 4; 4B). The operationally inactive 35S gene and bulk chromatin also give clear nucleosome ladders (Figures 3B tr. 3; 3C tr. 1; 4B), even in the small oligonucleosome region of the same gel tracks which show maximal smearing in the active galactokinase profile. Thus, both denaturing and nondenaturing gels show a gene specific disruption of the nucleosome structure on expressed galactokinase chromatin.

The nondenaturing gel profiles differ from the denaturing profiles in general appearance and in several specific features. Firstly, on denaturing gels all the residual peaks (tri-pentanucleosome and mono-dinucleosome) are similar in form, broadened and consisting of discrete bands. On the nondenaturing gels, there is heterogeneity in the extent of the disruption within the profile. While there are bands in the mono- and dinucleosome regions (Figures 3C trs. 2-3, 5-6; 4B), in the tri- to pentanucleosome region (Figures 3B tr. 2, 3C trs. 2-3, 5-6; 4B) and at higher nucleosome repeats ($>$ 5, Figures 3A, tr. 3, 4B), there is more extensive loss of the nucleosome repeat pattern. Secondly, the mainly diffuse mono- and dinucleosome peaks seen on non-denaturing gels (cf. Figure 3C, trs. 2, 3, 5, 6) resolve into sharp bands on denaturing gels (cf. Figures 2B, tr. 3, 2C, tr. 4; 2D, scan tr. 1). Thirdly, on denaturing gels the DNA between nucleosome peaks is present in a discrete band pattern. Even in extensive exposures on nondenaturing gels, the interpeak DNA appears diffuse (Figure 3C, tr. 3,5; others not shown). These denatured/nondenatured differences do not arise from trivial gel aberrations because the denatured/nondenatured profiles for inactive galactokinase and for 35S chromatin digests are similar (c.f. Figures 2C tr. 5/3C tr. 4; 2A tr. 1, 2B tr. 5/3B tr. 3, 3C tr. 1; others, not shown).

A possible (and the usual) explanation for different mobility profiles on denaturing and nondenaturing gels is that the DNA being analyzed is nicked. Nicks in digested DNA might also explain the somewhat anomalous nondenatured gel profiles from expressed galactokinase chromatin (Figure 3). Since electrophoretic resolution of DNA depends on molecular sieving, DNA conformation and flexibility will affect gel mobility. Internal nicks in double strand DNA seem likely to affect the flexibility, to an extent which may depend on the number and/or the location(s) of the nick(s), thus allowing a nicked DNA molecule on nondenaturing gels to exhibit various electrophoretic mobilities, versus the discrete mobility of an unnicked DNA

of the same molecular weight. The extent of the effect could also be molecular weight dependent, because the hydrodynamic behavior of DNA varies with its molecular weight (23). Without a more detailed theoretical understanding of DNA gel mobility, one cannot be certain that this explanation for the complex nondenatured gel profiles is correct. However, it does suggest that in these types of analyses, denaturing gel results are likely to portray the clearest picture of the molecular weight profile of a DNA sample.

There are two other observations which are consistent with this explanation. DNase I nicks bulk chromatin simultaneously in both spacer and within core particle DNA, which is somewhat analogous to the staphylococcal nuclease cleavage mode postulated here. In the literature there is data showing that electrophoresis of DNase I digests on nondenaturing gels can yield a profile resembling those in Figure 3, i.e. mono- and dinucleosome peaks but mainly smeared DNA at larger sizes (Figure 2, trs A-D, ref. 24; Figure 5, tr 7, ref. 25). Because DNase I digests relatively infrequently in the small yeast spacer, this experiment cannot be done in yeast (21).

The nondenaturing profiles of expressed galactokinase chromatin digests show less extensive smearing of the nucleosome pattern than the published profiles for active heat shock chromatin, done on agarose gels (1, 2). However, the effect of internal nicks on double strand DNA electrophoretic mobilities could also vary with the type of gel matrix. In agreement with this, active galactokinase chromatin patterns do show more uniform smearing on agarose (Figure 3D, tr 1). There is a very clear nucleosome repeat pattern when the gene is inactive (not shown) and a typical repeat pattern in the bulk chromatin profile for the same gel track (Figure 3D tr 2). Thus, the disruption of nucleosome structure on the active galactokinase gene is probably analogous to the disruption observed on the active heat shock genes (1,2) and it is likely that both have the same explanation.

DNase I Digestion Patterns

In contrast to staphylococcal nuclease, DNase I shows little discrimination for the spacer/core distinction. It nicks readily within both core particle DNA [at ~ 10.5 b intervals (21,26)] and spacer DNA [probably randomly (21)], at all times of digestion. Two regular, overlapping ladders of fragments, probably arising from the same set of intracore sites, are produced: up to 12 bands (126 b) resulting from two nicks solely within one core particle; from 120 b to at least 300 b, from 1 nick within each of two neighboring core particles (21). The interparticle

pattern can be distinguished from the intraparticle pattern, in the region where they overlap, because the presence of spacer DNA in 10 n + 5 bp sizes produces a 5 nucleotide increment in the former.

DNase I digestion of DNA-gyrase (27) and DNA-hydroxyapatite (28) complexes also produce regular DNA ladders, suggesting that such patterns reflect the accessibility constraints due to the binding of DNA to a surface (e.g. protein). Thus, DNase I digests can be used to monitor the DNA protein interaction in active versus inactive galactokinase chromatin.

Figure 5 shows the results. The gels shown include the upper few bands of the intraparticle ladder and much of the interparticle ladder. In contrast to the staphylococcal nuclease results, there are no striking pattern changes when the gene is expressed, neither in size nor in relative intensity of bands in either ladder (Figure 5, trs. 2, 5). Furthermore, there is little or no change in the very characteristic region where the two patterns overlap (inset, "{"), a diffuse region which always occurs between band #11 (intracore) and band #12.5 (intercore) and may or may not be resolvable into band(s) (21). The inset also shows that intracore bands #8-#11 have approximately the same relative intensities in active and inactive chromatin digests. The presence of regular patterns shows that expressed DNA is bound to a surface. That the transition from intra- to intercore patterns occurs in the same way in the same size region (band number 11-12.5) and that the higher ladder bands are the same size (i.e. include a 5 bp increment) suggests, within the structural predictive power of such ladder patterns, that the nature of the protein surface in expressed chromatin is unchanged, i.e., nucleosomal, for it seems unlikely that another set of proteins would constrain the same amount of DNA as the core particle (11 strong sites), in structures separated from one another by 10 n + 5 bp of DNA, just like the nucleosome spacer. The absence of changes in DNase I digestion patterns is not inconsistent with the staphylococcal nuclease pattern changes because DNase I cleaves throughout the core particle even in inactive chromatin (see Discussion).

Both the coding and noncoding strands (Figure 5 trs. 2,5) of several parts of the gene (i.e. Sau 3A a+b, d, f, Pvu II-RI) yield the same patterns, which are identical to the bulk pattern (Figure 5, tr 1). The weaker signal of the pattern from galactose grown cells reflects only a lower loading of DNA because both the gene specific and the bulk pattern show the same decrease (Figure 5, trs. 1,2). This data also confirms a previous conclusion that active yeast chromatin is not preferentially DNase

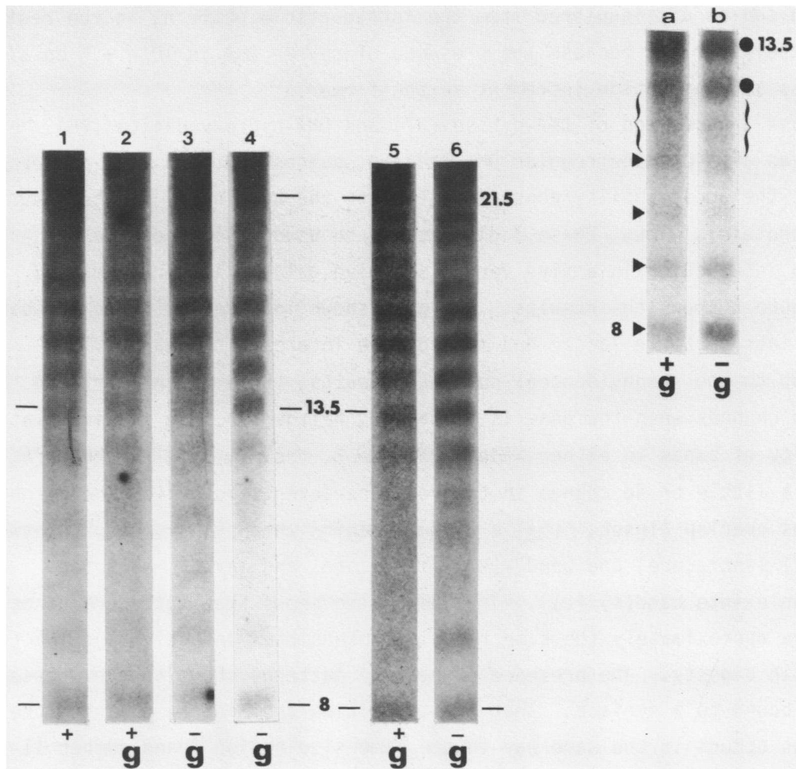

Figure 5. DNase I Digestion Patterns of the Galactokinase Gene in Various States of Expression

DNA from DNase I digests of nuclear chromatin from cells grown in various carbon sources was electrophoretically separated, transferred to DBM paper and hybridized with probes from the galactokinase gene or bulk DNA. The positions of some representative bands are shown by band number. Band number refers to the 14 bands obtained by a DNase I digest of core particles (e.g., # 8 = 84 b or 8/14 of a core particle DNA length). The half integral bands, e.g. 13.5, reflect the 5 b increment in the yeast extended ladder bands. Electrophoresis is from top to bottom. The state of galactokinase gene activity in the cells from which the chromatin was isolated is indicated immediately below the track by "+", the gene is expressed (log phase in galactose) and "-", the gene is repressed (log phase in glucose) or quiescent (stationary phase in galactose). In the second row below the track, a "g" indicates that a galactokinase specific probe was used. The Sau3A a+b probe used is specific for the non-coding strand while the Pvu II - RI probe is specific for the coding strand.

Gel Track	Growth State/ Carbon Source	Galactokinase Gene	Probe
1	log / galactose	+	bulk DNA
2	" "	+	Sau3A a+b
3	" / glucose	-	"

4	stationary / galactose	-	"
5	log / "	+	Pvu-RI (250 bp)
6	" / glucose	-	"

The inset shows part of the pattern from another DBM paper probed with Pvu-RI (250 bp). The "+" track shows a digest from log/galactose cells while the "-" track shows a stationary/galactose digest. This paper shows the lower ladder bands #8-#11 ("▶") and the distinctive transition region ("}") between the intracore ("▶") and intercore ("●") patterns.

I sensitive (29), for there is no evidence of enhanced digestion rate of expressed galactokinase chromatin, by comparison to inactive galactokinase chromatin (Figure 5) or, for the same gel tracks, to bulk chromatin (Figure 5) or 35S rDNA (not shown).

DISCUSSION

Both denaturing and nondenaturing gels show clearly that active expression of the galactokinase gene results in a loss of the typical nucleosome ladder pattern seen in staphylococcal nuclease digests. These results thus generalize the reversible smearing in the chromatin digestion profile of an expressed gene, first noted on the heat shock genes (1,2), and extend those observations by making suggestions about the structural basis of this disruption. The similarity of nuclease patterns on both strands argues against histone octamer transfer to one strand (30). The occurence of the usual DNase I ladder patterns suggests that the same types of DNA/histone surface interactions are present in expressed and inactive chromatins. This plus the maintenance, in the denaturing profiles, of some enhanced intensity at precisely the usual nucleosome repeat positions, probably corresponding to spacer DNA cleavage, plus the reversibility of the structural change combine to suggest that histones remain and that the nucleosome may simply undergo a reversible conformational change when the gene is expressed.

Staphylococcal nuclease digestion of typical nucleosomal chromatin produces a ladder pattern because the nuclease digests in spacer DNA, mainly avoiding the core particle domain, at least until late in digestion. The avoided domain provides the DNA present in the nucleosome repeat peaks. There is little DNA between these peaks, since in the absence of intracore cleavage there is no way to produce such DNA. The increased interpeak DNA in the expressed galactokinase profiles, particularly on denaturing gels, thus suggests that the conformational change on the expressed gene increases

the accessibility of the normally resistant core DNA. The susceptibility increases are substantial, for a significant amount of DNA is liberated into the interpeak region. The observation that DNase I shows no pattern changes is in agreement with this suggestion, for even in inactive chromatin DNase I digests readily within the core particle. The entire core particle domain must become available to the nuclease (Figure 2D, tr. 1).

The increased intracore digestion seems to occur primarily by nicking rather than double strand cleavage. The nicking cannot result from consistent structural differences between the coding and noncoding strands, because the same patterns are produced from digestion of each strand. The nicking may simply arise because, at a core particle staphylococcal nuclease recognition site (22) ,the two strands may be differentially protected by protein, making it unlikely for a second phosphodiester interruption to occur opposite the first, at least under conditions of limited digestion. For example, even in extensive staphylococcal nuclease digests of bulk yeast chromatin, when there is considerable digestion within the core particle, there is evidence for nicks in subcore particle DNA (Lohr, unpublished observations).

Other possible interpretations of the data were considered. For example, the disorganization when the gene is expressed could involve only a chromatin region upstream (or downstream) of the gene. However, probes from the 5' end or the middle of the gene give the same pattern. Also, there is clearly disruption of tri-pentanucleosome length DNA on both denaturing and non-denaturing gels. Since the Pvu II-RI probe is 850 bp from the 5' AUG codon and 720 bp from the termination codon, it would not be possible for a tetranucleosome (4 x 170 = 680 bp) length (or smaller) DNA fragment in these profiles to have <u>any</u> homology with noncoding sequence DNA. For the striking disruption patterns in the mono- to dinucleosomal region on denaturing gels, the DNA is small enough that most of the signal must come entirely from sequences homologous to the probe. Thus, when the gene is expressed, there must be disruption of nucleosomal structure on the coding sequence chromatin itself (and perhaps extragenically as well).

Histone sliding to generate nonnucleosomal DNA is another possible explanation for the smeared pattern. However, the maintenance of the usual DNase I ladder, the presence of some enhanced intensity at the usual repeat positions (corresponding to spacer DNA cleavage) and the observation that the newly produced DNA in the staphylococcal nuclease profiles, the "interpeak" DNA, is present in a discrete band pattern rather than as a

smear seems inconsistent with this explanation.

It is also possible that some, as yet uncharacterized, property of expressed DNA itself is responsible for the smeared staphylococcal nuclease electrophoretic profiles. However, if the anomalous migration were caused by a change in the DNA, then the DNase I profiles should also show an alteration in the active gene digests. Thus, the explanation offered above, intracore digestion (nicking) of expressed chromatin by staphylococcal nuclease, seems most consistent with the data and is therefore favored.

One way to increase the accessibility of core DNA to staphylococcal nuclease is to expose the core domain by unfolding the particle. A simple loosening of the DNA-histone interaction seems less likely, for such loosening should affect the DNase I pattern, which assesses the DNA-histone interaction more directly, and also would not be expected to promote nicking over double strand cleavage.

There are experimental precedents for conformational transitions in core particles. For example, the core particle undergoes a conformational unfolding transition(s) _in vitro_ at low ionic strength, which involves a substantial opening of the particle, into a shallow one-turn helix (31). Although relative histone movement accompanies this transition (32), it does not involve loosening of the DNA from the histones because the usual histone DNA contacts are maintained in the unfolded particle (33). Secondly, an unfolded structure has been observed for SV40 nucleosomes (4). Finally, the model which reflects the current understanding of the protein-DNA interaction in core particles (34) suggests a number of possible pathways to achieve such unfolding while maintaining histone-DNA contacts (c.f. 33; Van Holde, private communication). Thus, core particle unfolding transitions are viable structural possibilities for active chromatin structural changes.

Such a conformational transition could have topological consequences. Relaxation of the core particle with its $1^{3/4}$ turns of toroidally supercoiled DNA into a less constrained structure, for instance, containing 1 turn as suggested above, would result in a change in the writhe of nucleosomal DNA, in this example, an increase from $-1^{3/4} \rightarrow -1$ ($= +3/4$). If there exists a topological constraint which prevents free rotation of the two strands about one another, as has been suggested (c.f. 35; yeast, 36), then within this constrained domain any writhe increase must be compensated for by a twist decrease, to maintain an invariant linking number within the domain.

A decrease in twist, associated with a nucleosome unfolding transition,

could aid transcription. Although the magnitude is uncertain, if one uses the example above, $\Delta Tw = -3/4$, spread evenly over 146 bp of core particle DNA would convert it all from 10.5 bp/turn to 11.0 bp/turn, the pitch of A form DNA, the form observed in triple helices and in DNA-RNA hybrids. If this $-\Delta Tw$ were localized, about 7-8 bp per nucleosome could be completely unwound. By simultaneous unfolding in adjacent nucleosomes, the amount of DNA unwound would be comparable to the amount of DNA estimated to be unwound during E. coli transcription (39). In this regard, it is interesting that the quantitative level of supercoiling maintained in E. coli DNA (38), which is thought to play a role in the transcription process, equals that provided by histones. Certainly the conformational transition discussed here is a hypothesis and the magnitude of ΔTw which might be associated with such a transition uncertain. Nevertheless, the fact that this model contains features which could aid in carrying out processes likely to be involved in transcription does fulfill a criterion which is important for any model of expressed chromatin.

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